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JOURNAL OF HYGIENE

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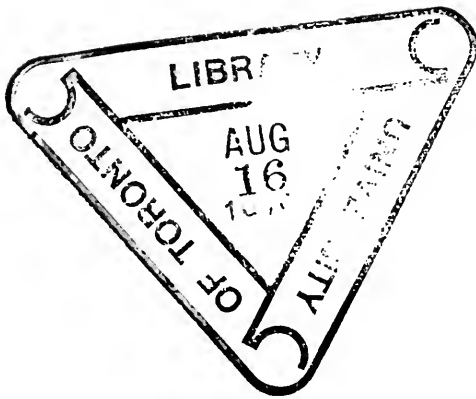
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ON THE DANYSZ EFFECT.

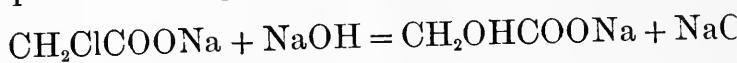
BY SVANTE ARRHENIUS.

10 IN this *Journal* (vol. VII. p. 501) Mr J. A. Crow has recently published a very sharp criticism of¹ my calculations based on experiments by Madsen and Walbum regarding the so-called Danysz Effect.

Before entering upon the objections of Mr Crow it appears advisable to recall some points in the development of this question. The effect found by Danysz² in experiments with ricin and diphtheria-toxin in 1900 was demonstrated by von Dungern³ and Sachs⁴ for some other toxins and was by these authors considered inexplicable, from the point of view taken by Madsen and myself, that the neutralisation of a poison by its antibody is a chemical process subject to the law of mass-action. To explain this effect von Dungern supposed the existence of a new substance called epitoxonoïd in the diphtheria poison, which even before had been supposed by Ehrlich to contain some seven or eight different substances, by the presence of which Ehrlich tried to explain different phenomena observed by himself and his pupils. Sachs also participated in this opinion regarding the constitution of the poisons investigated by him.

As very few exact data were published regarding the said effect and it was held by the Frankfort school that it was inexplicable from our standpoint, Madsen executed, with the assistance of Walbum, a great number of experiments regarding the behaviour of tetanolysin in this relation, and gave them to me for calculation. It is against this calculation that Mr Crow directed his criticism. In order to clear up the question it will be useful for the sake of comparison to give an example of a chemical reaction, analogous to the effect of Danysz, and

This example regards the chloracetic acid which has been investigated by Schwab¹ in the laboratory of van't Hoff. If a quantity of acid is neutralised with an alkali, *e.g.* sodium hydrate in excess, the reaction takes place according to the formula:



indicating the formation of glycolic acid from the chloracetic acid. The reaction goes on very slowly at room temperature, but at temperatures above 65° C. We will therefore suppose that the reaction takes place at this or higher temperatures.

The alkalinity of the mixture decreases, at first more rapidly and then on more slowly, and the rate of decrease is proportional to the quantity of free sodium hydrate (or better hydroxylions) and to the quantity of sodium chloracetate present. After a sufficient time (about 100° C.) the sodium hydrate is neutralised, if the chloracetic acid is in excess. The limit quantity of neutralised sodium hydrate is simply proportional to the quantity of sodium hydrate used up for neutralising the chloracetic acid.

Suppose now that we have two solutions, the one containing one gram molecule of chloracetic acid and the other two gram molecules of sodium hydrate; if we mix them at once, two gram molecules of sodium chloracetate are formed, and thereafter no sensible reaction takes place. The solution is neutral. If on the other hand we divide the sodium hydrate solution into two equal parts and mix the one of them with the chloracetic acid solution and let the mixture stand at 100° C. for some time, and then after add the rest to the acid, the resultant mixture will be neutral. This the more, the longer time the first mixture has been heated. After a sufficient time the first mixture will have reached a certain degree of acidity, it will be neutral, so that the whole quantity of acid will remain unneutralised. If we call the acidity of the part of the original two solutions 2, then the acidity of their mixture at once will be 0, and the acidity of the fractionated mixture will increase with the time during which the first fraction has been heated at 100° C., and tend to a limit value, in this case 1. If we have a solution of sodium hydrate containing more than one and

chloracetic acid in the first fraction, or, in other words, proportional to the quantity of free alkali in the first fraction.

Evidently this phenomenon shows a very marked parallelism with the effect of Danysz. There the chloracetic acid is represented by the toxin, the alkali by the antitoxin, and the toxicity by the acidity. If we take equivalent quantities of toxin and antitoxin in two solutions and divide the toxin solution in equal parts, the quantity of free toxin required is greater if we mix the one part of the toxin with the antitoxin, and let it stand for a time and thereafter add the rest of the toxin, than if the total quantity of toxin is mixed simultaneously with the antitoxin. There is one difference, but only a quantitative one, namely that in a mixture of equivalent quantities of toxin and antitoxin there is always a certain quantity of free poison present, just as there is free boracic acid in a mixture of equivalent quantities of ammonia and boracic acid. In other words, the reaction between toxin and antitoxin is incomplete just as that between ammonia and boracic acid, whereas the reaction between alkali and chloracetic acid is said to be complete. But every chemist knows that this is not exactly true, since the sodium chloracetate is hydrolysed to a certain, although very slight, extent. If now in the first fraction there is free antitoxin (as is always the case), the limit value of the Danysz Effect is proportional to this quantity of free antitoxin, just as the limit value of the corresponding neutralising effect of the chloracetic acid is proportional to the quantity of free sodium hydrate. But as the proportionality for this later effect is only valid till the quantity of free sodium hydrate reaches a certain limit, the same will probably be true for the Danysz Effect, but this point has not as yet been investigated.

Now Mr Craw says that I am not entitled to suppose that the Danysz Effect tends to a certain limit with increasing time of reaction of the first fraction. If this were not true, we might suppose that we would obtain an infinite effect, *i.e.* an infinitely great toxicity of the mixture, if the time of reaction increased indefinitely. That would be just as right as to suppose that the acidity of the chloracetic acid mixture would increase to infinity with increasing time of reaction of the first fraction. It seems to me unnecessary to enter into a more detailed discussion of this point.

quantity of toxin. I have only assumed that the effect is not sensible, *i.e.* does not exceed the errors of experiment in the said case. To show this proportionality in the most evident manner, I give a little table below, cited in our memoir p. 13, in which I have calculated the effect as proportional to the quantity of antitoxin present in the experiments with a constant quantity of toxin and varying quantities of antitoxin. The basis of the calculation is that 0.18 c.c. of antitoxin are equivalent to 1 c.c. of toxin and that the equilibrium constant is $K=0.131$ at 37°C. , as had been determined through other experiments on the neutralisation of toxin with antitoxin (*cf.* p. 5). In this manner I find :

Composition of the first fraction	Quantity of free antitoxin		Danysz Effect	
	In per cent.	Total	Observed	Calculated
0.2 c.c. antitoxin + 1 c.c. toxin	30.3	0.0606 c.c.	5	5.7
0.4 „ „ + 1 „	58.6	0.234	23	22.1
0.6 „ „ + 1 „	71.2	0.427	39	40.9
0.8 „ „ + 1 „	78.3	0.626	60	59
1.2 „ „ + 1 „	85.3	1.024	97	96.4

The agreement between the two last columns is excellent and far within the errors of observation, and it may well be concluded that this series of observations gives a “very strong support” to the correctness of Madsen’s and my idea that the laws of mass-action are valid. Evidently the determination of the quantity of antitoxin, which is equivalent to 1 c.c. of toxin by means of these last experiments, gives precisely the same value as the direct determination. This corresponds to the possibility of calculating the quantity of free sodium hydrate present in the mixtures, used in Schwab’s experiments, from the end-value of the acidity as well as from the direct measurement of the neutralising quantity.

The effect of Danysz, which had been employed as an argument against the use of the laws of mass-action on the reactions between antibodies, has by this calculation given the most startling proof of the applicability of these laws. It should be remembered that *the opponents of the use of these laws have still given no explanation at all of the Danysz Effect, especially of the experiments cited in our memoir.*

Mr Craw asserts that the law of mass-action, which I have applied to these phenomena, gives an equation which should only be regarded as an interpolation formula, because it contains two constants which may be determined from the experiments. It may well be remembered that I have found this formula applicable in all the thirty-five different

cases which I have treated in my "Immunochemistry¹," and which are taken from all the different departments of immunity. On the other hand Mr Craw asserts with Mr Biltz, that the neutralisation phenomena of toxin with antitoxin are due to the "adsorption" of the toxin by the antitoxin. "If m is the quantity of adsorbing matter, x the adsorbed quantity of the dissolved substance, c the concentration of this substance, after the adsorption process has reached its limit value, α and p are constants," then, says Freundlich², "the following equation holds good:

$$\frac{x}{m} = \alpha c^p,$$

where p in nearly all cases investigated falls between 0.1 and 0.4."

It is possible to apply this formula, which, as well as that deduced from the law of mass-action, contains two constants α and p which are determined from the experimental results, to the great material collected in my lectures on immunity. It may well be regarded as very peculiar that the seven authors enumerated by Mr Craw as adherents of the adsorption theory, have not used this opportunity of proving the correctness of their theoretical views. I have tried to make this examination in six of the best investigated cases of neutralisation of toxin with antitoxin and find that p has no constant value. With tetanolysin for instance p varies from 0.235 to 0.77 with a mean value of about $\frac{1}{3}$. If we calculate the data for tetanolysin with this mean value, we find the figures written below under T_{calc_2} , which ought to be compared with the values T_{calc_1} found by application of the formula given for the mass-action and the directly observed values T_{obs} , giving

n	T_{obs}	T_{calc_1}	T_{calc_2}
0	100	100	100
0.05	82	82	82
0.1	70	66	66
0.15	52	52	53
0.2	36	38	42
0.3	22	23	25
0.4	14.2	13.9	16
0.5	10.1	10.4	10.0
0.7	6.1	6.3	4.5
1.0	4.0	4.0	1.7
1.3	2.7	2.9	0.8
1.6	2.0	2.5	0.4
2.0	1.8	1.9	0.2

¹ *Immunochemie*, übers. v. Finkelstein (Akad. Verlagsgesellsch. Leipzig, 1907); *Immunochemistry* (The MacMillan Co., New York, 1907).

² H. Freundlich (1907), *Zeitschr. f. Chemie u. Industrie der Kolloide*, 1. 325.

the quantity of free tetanolysin after the addition of n parts of anti-tetanolysin (cf. *Immunochemie*, p. 117, English ed. p. 178).

An analogous calculation is given below for the neutralisation of rennet. In this case we know the probable errors, which are written under Δ (cf. *Immunochemie*, p. 180, English ed. p. 275), $p = 0.05$.

n	T_{obs}	T_{calc_1}	T_{calc_2}	Δ
0	100	100	100	—
0.02	97.4	97.1	97.0	0.6
0.05	92.3	92.6	92.5	1.4
0.1	85.9	85.2	85.1	1.5
0.2	70.4	70.6	70.4	1.8
0.3	54.3	56.0	56.1	1.9
0.4	42.6	41.8	42.3	1.3
0.5	30.2	28.2	29.2	1.2
0.6	16.5	16.3	17.2	0.4
0.7	8.2	8.4	7.4	0.6
0.8	4.7	4.7	1.7	0.3
0.9	2.8	3.1	0.2	0.2

The adsorption formula does not give nearly so good results as the mass-action formula, and the discrepancy between T_{calc_2} and T_{obs} is startling, especially for the higher values of n . There the difference between these two values exceeds the probable error ten times or more, which may be regarded as proving the impossibility of the adsorption hypothesis (cf. *Immunochemie*, p. 142, English ed. p. 216).

Now Mr Craw repeats the objections of the Frankfort school, that the Danysz Effect upsets the view of simple equilibrium which is adopted as the basis for my calculation. It may be noted that in the memoir to which I have referred, I have shown "that this disturbing action is without sensible influence at 20° , if the time of reaction does not exceed one or two hours, a condition to which we have adhered in our experiments." This may easily be verified with help of the figures given in the said memoir, and Mr Craw has not tried to raise an objection against those figures. That it is necessary to avoid using too long periods of reaction in working with tetanolysin was already known to Madsen and myself in 1900, when we carried out the determinations for our first memoir¹ on this toxin. The tetanolysin weakens with time, but our experience showed us that *this deterioration was negligible during the time occupied by our experiments on the Danysz Effect*. Different preparations are very unlike in this respect, so that Mr Craw's observation that a 4 % solution of tetanolysin lost 25 % of its activity in one hour

¹ *Festskrift ved indvielsen af statens serum-institut*, No. 3, Copenhagen, 1902.

at 37° C. may well be possible—perhaps the experimental error has contributed to the high figure.

Mr Craw is opposed to my treatment of the Danysz Effect as depending on a monomolecular process. This conclusion was based by me on the observation that the magnitude of the effect is independent of the concentration of the reacting substances, and not at all on the progress of the reaction with time. The experimental errors are in this latter case too great to permit a definite conclusion, but *the experiments on the influence of concentration seem to me to be wholly convincing*. Mr Craw seems to have overlooked these experiments. He calculates the figures on the time progress as if the process were bimolecular and finds the agreement with the observation “highly satisfactory.” The mean difference between observed and calculated values is according to my calculation, assuming the process to be monomolecular, 1.8%, and according to Mr Craw’s calculation on the assumption that the process is bimolecular, 1.6%, so that the two calculations may be said to be of very nearly the same value. Mr Craw therefore might regard the hypothesis that the process is monomolecular as being “highly satisfactory.” This supposition that the process is bimolecular seems to me to be incompatible with the experiments on the influence of concentration.

There is still a point of theoretical interest, on which Mr Craw lays great stress. He is of opinion that colloïds, among which he reckons the antitoxin, do not diffuse. If the antitoxins were diffusible they would exert an osmotic pressure, and then the laws of equilibria might be applied, as I have done¹. Now Madsen and I² showed that antitoxins diffuse in a weak solidified solution of gelatin (5%), and even that the distribution of the antitoxin conforms to the general laws of diffusion, formulated by Stefan. Mr Craw says that our experiments do not show that antitoxin diffuses; the presence of the 5% of gelatin caused the apparent diffusion, but the phenomenon observed was really due to imbibition, which is supposed not to exert its influence in similar experiments of Mr Craw. I confess that I cannot understand Mr Craw’s argument. The difference of our opinions seems of little importance since Herzog and Kasarnowski have found that colloïds in general are subject to the general laws of diffusion. Herzog gave a lecture on Kasarnowski’s experiments at the meeting of the Bunsen-Gesellschaft in Hamburg and drew from them the following conclusion: “The values found indicate that the osmotic pressure is the driving force in the

¹ Cf. *Immunochemie*, p. 19, English ed. p. 28.

² *Festskrift*, No. 4, Copenhagen, 1902; *Immunochemie*, p. 16, English ed. p. 25.

diffusion of the colloïds investigated¹." Nernst and Biltz, who are cited by Mr Craw as holding the opinion that colloïds do not diffuse, were present and took part in the discussion after the lecture, but they raised no objection to the view that the diffusion of colloïds is due to their osmotic pressure.

It is much to be regretted that the authors, who support the adsorption hypothesis, do not use it for calculation of the great number of observations collected by different observers, especially those of Madsen and his collaborators. They would thereby contribute much more to the clearing up of the ideas on the nature of the action of antitoxins than they have done by their general assertions.

¹ *Zeitschr. f. Elektrochemie*, 13, 1907.

SUPERSENSITIZATION TO ALIEN SERUM¹.

By ALBERT S. GRÜNBAUM, M.D., F.R.C.P.

(*From the Pathological Laboratory, University of Leeds.*)

THE phenomenon of supersensitization observed on repeated injection of alien sera has lately aroused much experimental and clinical interest. The following cases which led me some three years ago, on clinical grounds to assume the occurrence of this phenomenon, appear worth recording, especially since the reaction happened not with anti-diphtheritic or anti-plague but with anti-tuberculous serum.

The reaction occurred in a series of cases treated with the same supply of slightly blood-stained serum and the variation in the results is likely, in my opinion, to be due nearly as much to the presence or stage of the disease as to any idiosyncrasy on the part of the patient.

The histories will show that there was nothing especially and immediately toxic in the serum, but it may be as well to state that the serum was tested after each accident by single injections, on both guinea-pigs and monkeys, without any bad symptom or untoward result.

The serum had been tried in eleven cases, when the occurrence of a death attributable directly to the serum made further administration inadvisable.

In no instance did the *second* injection produce any alarming or uncomfortable effect; the *fourth*, in one case, was followed by urticaria and other subjective symptoms, on account of which the patient refused further injections; in another case oedema of the tongue and larynx followed two successive injections and the administration was therefore stopped.

Concerning the following five cases more details are given because although the number of injections was large, either no accident happened or it happened suddenly without warning—at any rate the patients had made no previous complaint. The serum was given in series on successive or alternate days for several doses, followed by an

¹ Portion of a paper read before the Leeds and West Riding Medico-Chirurgical Society on 11 December, 1906, with references to later literature added subsequently.

interval of several days before the commencement of the next series. It will be noticed that the bad effects always occurred in the course of a series and not at the commencement.

W. C., male, aet. 40. This man's temperature was generally under 99° F. and tubercle bacilli were alleged to have been found on one occasion in the sputum. He had absolutely no symptoms from the serum and left the hospital cured.

1904.	
Jan. 19... 8 c.c.	Feb. 6 { 16...5 c.c.
22... 8	22...4
25... 8	23...5
27...10	24...5
30... 9	25...5
Feb. 1...10	26...5
9 { 2...10	Mar. 8 { 27...5
11... 5	7...5
12... 2.5	8...4
13... 5	

19 injections=118.5 c.c.

A. H., male, aet. 25. A case of advanced phthisis with a temperature varying at least three degrees in 24 hours and sometimes six degrees. He died on 16 March, 1904. No symptoms attributable to the serum occurred.

1904.	
Feb. 1...5 c.c.	Feb. 11...6 c.c.
2...5	12...5
4...5	13...5
5...7	6 { 16...5
8...8	22...5

10 injections=56 c.c.

The following cases developed serious symptoms.

R. W., male, aet. 31. A case of phthisis in the third stage, with pyrexia which increased with the injections. He also had an ankylosed hip-joint. Almost immediately after the last injection, he complained of "feeling queer," became cyanosed, vomited, lost consciousness and died within five minutes.

1904.	
Jan. 20...7 c.c.	Feb. 6 { 16...5 c.c.
22...7	22...4
23...7	23...5
25...7	24...5
26...6	25...5
30...9	26...5
Feb. 9 { 2...9	Mar. 8 { 27...5
11...9	7...5
12...5	8...5
13...5	9...5

20 injections=116 c.c.

M. S., female, aet. 41. A case of phthisis in late second stage. The temperature reached a higher general level during the serum administration than before and rose yet higher after its cessation. She was discharged, certainly no worse, on 14 March.

About two minutes after injection of serum on Feb. 27 the patient began to feel faint, felt blood rush to head, had sensation of choking and thought she was becoming blind. Objectively, she became suffused a deep bluish red, the eruption appearing on the fore arms and thighs as well as in the face. Subsequently she vomited. Pulse became rapid, 120, and remained so for 24 hours. Pain in front and back of chest for a day.

1904.	
Jan. 25...5 c.c.	Feb. 16...5 c.c.
27...5	5 { 17...5
30...5	
Feb. 1...7	23...4
2...9	24...5
4...9	25...5
5...9	27...5
8...5	
9...5	
11...5	
14...5	18 injections = 103 c.c.

S. M., female, aet. 22. A case of second stage phthisis with pyrexia to 100° F., rising higher during a few days of the serum administration period with some improvement later.

Only 2 c.c. under the left scapula had been given to this patient when the symptoms arising in the immediately preceding case were observed and the injection consequently stopped. Nevertheless after two minutes patient felt tightness at throat, saw "funny things," was then unable to see and finally lost consciousness. She lay back in bed, flat, waving her arms about with eyes turned upwards and twitching of the face. This condition lasted about a minute. With returning consciousness she complained chiefly of pain in the head, with some tightness and pain on right side of chest, back and front, like the preceding case. She mentioned then that she had had similar pectoral pain after the previous injection. Pulse quick and irregular. Recovery rapid except pulse which remained quick for 24 hours. Discharged improved on 22 March.

1904.	
Jan. 27...2 c.c.	Feb. 6 { 16...5 c.c.
30...5	6 { 22...1
Feb. 1...7	
3...6	23...4
4...6	24...5
5...8	25...5
9...5	27...2
11...5	
13...5	
15...5	16 injections = 76 c.c.

I record these cases chiefly to add to the necessary array of facts required before we can theorize about the phenomenon. They do not, it seems to me, fit in well with any hypothesis yet put forward, but they tend to diminish the importance of the element of time and to emphasize that of accumulation.

There are two possible factors which have not received much attention in the explanation of the phenomenon: the one is the constitution of the serum, the other the condition of the patient.

So far as anti-diphtheritic serum is concerned, Anderson and Rosenau believe, from their experiments, that antitoxin plays no part. Its effect may have been inappreciable in their experiments although it must be remembered that normal horse serum contains some antitoxin. But they also found that the presence of some toxin appears to increase the supersensitiveness. Consequently while the reaction depends mainly no doubt on a substance peculiar to the alien serum, it may be assisted by the presence of toxin, perhaps neutralized in the serum as injected, but subsequently dissociated in the body. A serum may be antitoxically very potent and yet contain some neutral mixture, and possibly some such dissociation explains in part the relatively slight effect of anti-diphtheritic serum in preventing or mitigating paralysis.

In anti-tuberculous serum it is more than likely that the antitoxin is minimal in amount and that the curative action is largely due to a minute quantity of tuberculin. (The fact that the serum has been found to do good when tuberculin has failed, does not negative this supposition because of the difference in dose.) But the presence of tuberculin may help to induce the phenomenon of "supersensitization" and by its continuous injection produce a deep negative phase of diminished resistance. The alien serum itself acts also like a vaccine so that each succeeding administration, taking place probably during a negative phase, leads up to the final catastrophe.

To a tuberculous patient in whom auto-inoculation is taking place constantly, this additional small dose of tuberculin may make no difference. Nor would it affect a non-tuberculous patient. In this way I explain the indifference of the first two cases above recorded.

Probably the chief factor is personal idiosyncrasy, and this can be determined only by trial. Two rabbits of the same breed may be injected intravenously with 10 c.c. of the same human serum, and one will remain unaffected, while the other will be dead in ten minutes. Again ordinarily harmless substances like eggs, sole, chocolate or strawberries produce toxic effects in some individuals, so that it is

not remarkable the generally harmless horse serum occasionally produces the same.

Under all circumstances I believe considerable care should be bestowed on cases treated with repeated injections of alien serum, and the injections should be intermitted for a period, as long as compatible with the safety of the patient, as soon as the first sign of "serum disease" appears. In all cases where rectal administration is efficient, it should be preferred to subcutaneous injection.

Had Anderson and Rosenau's experiments with animals been published before the cases related above were treated, some of the accidents might have been avoided.

REFERENCE.

- CURRIE, J. R. (II. 1907). On the supersensitization of persons suffering from diphtheria by repeated injections of horse serum. *Journ. of Hygiene*, vol. VII. p. 35. (References to all the important literature are given.)

ON SOME NON-SPECIFIC REACTIONS OF MALLEIN.

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(8 Charts.)

It has always been held by the veterinary profession that the mallein reaction is absolutely specific, although Schattenfroh in 1894¹ claimed to have shown, as the results of some tests upon guinea-pigs, that mallein acted similarly to various bacterial proteins. In all cases the reaction was indicated by temperature since no swellings were obtained in guinea-pigs. The preparation (Foth's mallein) used by Schattenfroh was merely the proteins precipitated by alcohol from concentrated broth cultures of *B. mallei*, so that his conclusions do not apply to mallein as it is now prepared.

It has been recognised for some time that mallein causes a local swelling on horses suffering from purpura, but there is no rise in temperature, nor is the swelling a typical glanders reaction but disappears much more rapidly. The double reaction of both temperature rise and local swelling has been taken invariably to indicate glanders, although in some cases the local swelling alone is sufficient to condemn a horse.

The small rapidly disappearing swelling produced in normal horses has recently been used at these laboratories as an indication of the relative strengths of different samples of mallein. Owing to a lack of normal horses, a test² was made of a fresh sample of mallein upon a healthy horse which had undergone treatment with diphtheria toxin for

¹ "Über die Wirkung von Bakterin-Proteinen," *Zeitschr. f. Hygiene*, Bd. xviii. p. 456, 1894.

² The injections were all performed by H. J. Südmersen.

over two years. This horse was chosen because at the time it happened to be resting after a period of treatment. The writers were greatly surprised to obtain a large local reaction and a rise of temperature sufficient to condemn the horse (see Chart I).

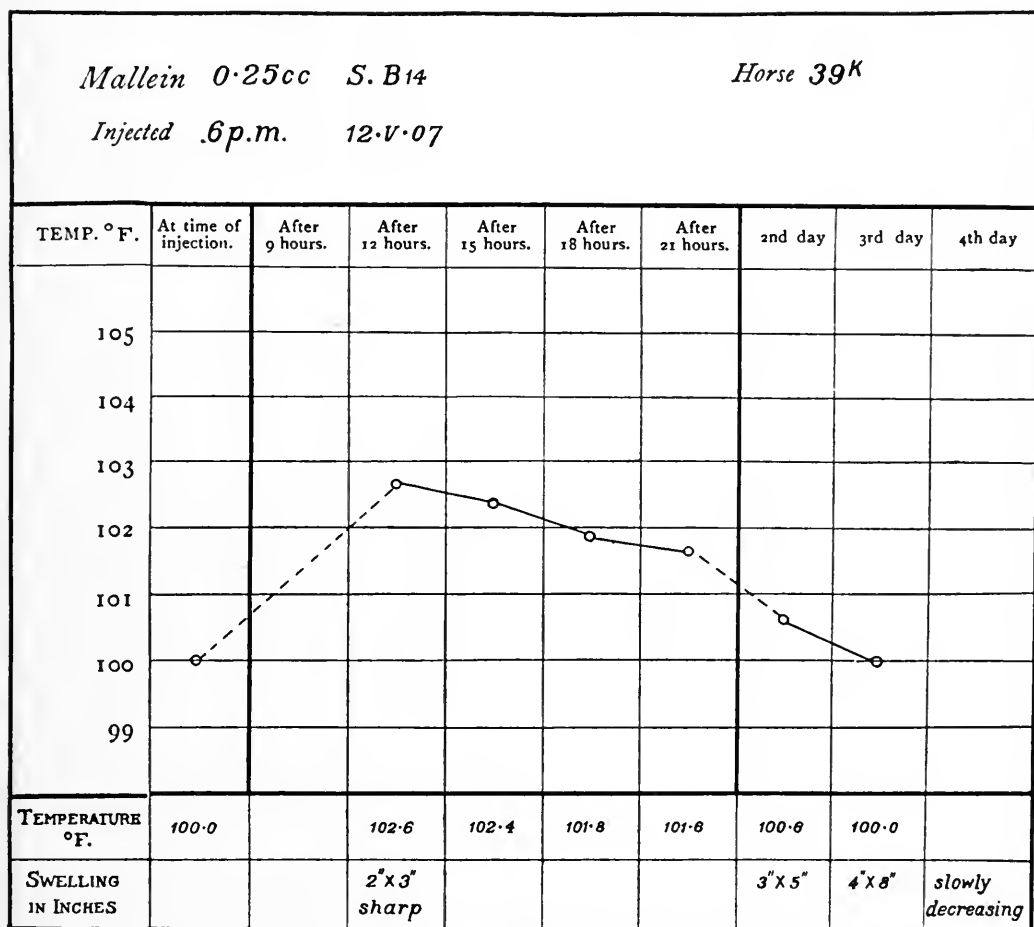


Chart I. First mallein reaction of horse 39K.

As the horse was well immunised and produced valuable antitoxin, it was decided to repeat the injection before condemning the animal. A second dose of 2 c.c. mallein (equivalent to twice the former dose) was injected within three days of the first injection. The local swelling produced was more marked than before and the temperature reaction was similar (see Chart II). After both injections of mallein the horse was "off his food and in low condition."

The reactions obtained were considered sufficient to condemn the horse as glandered, and on May 17th the horse was destroyed. Post-mortem examination was undertaken by Messrs Hunting and Humphrey, Veterinary Inspectors to the London County Council, but no trace of glanders could be found. On the 14th, cultures had been taken directly

from the blood of the horse into broth, and on to agar and Loeffler slopes, but no growth took place. Two mice were injected with 0.5 c.c. each of fresh serum, and two guinea-pigs of 700 grams weight received 2 c.c. each, and no ill effects were observed in any of the animals.

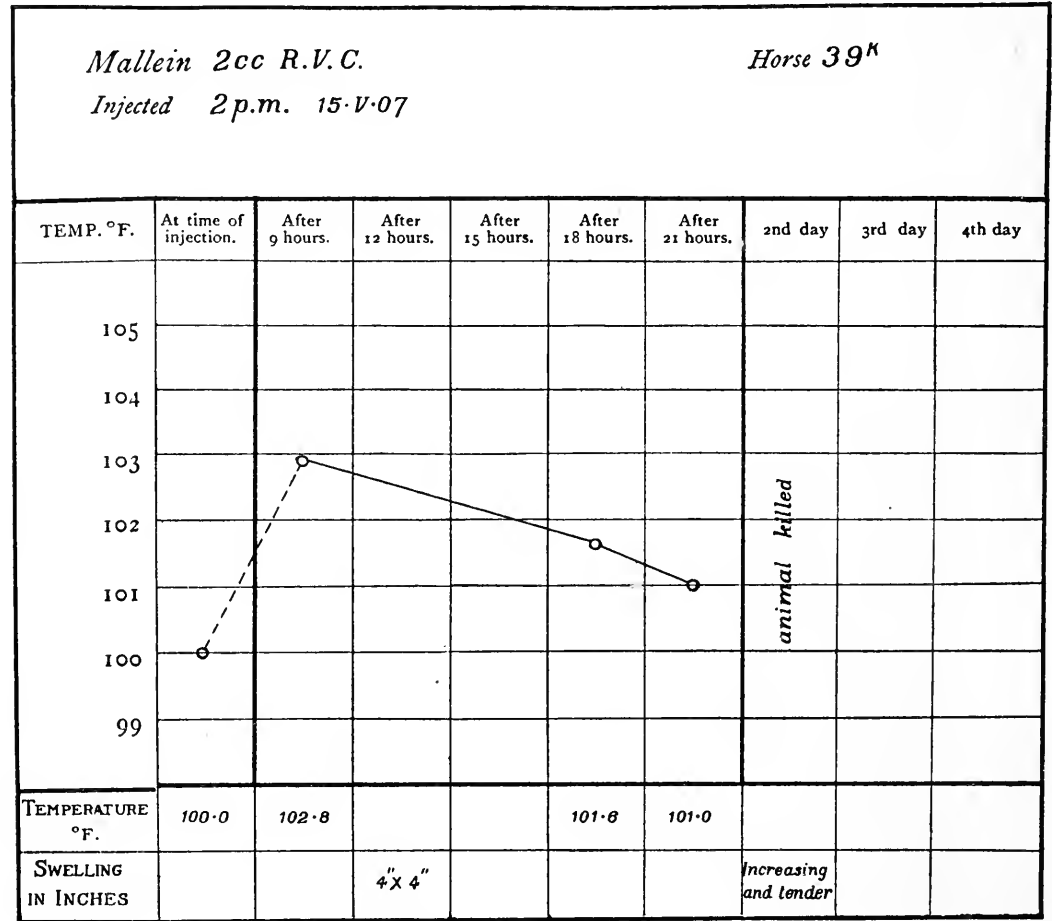


Chart II. Second mallein reaction of horse 39K.

On comparing the temperature curves of the foregoing horse (39K) with the average curves obtained from 19 horses (tested before admission to the stables), which gave both the local and temperature reaction and which, on post-mortem, were found to be glandered (see Chart III), it will be seen that the maximum rise in temperature in the case of horse 39K corresponded exactly with the average rise in definitely glandered horses.

Comparing Charts I and II with Charts III and IV (the latter giving a typical temperature reaction for a glandered horse) it will be noted that the general slope of the curves is different. In glandered horses the highest temperature is apparently reached 15 hours after the injection of mallein and the fall in temperature is gradual. In the case

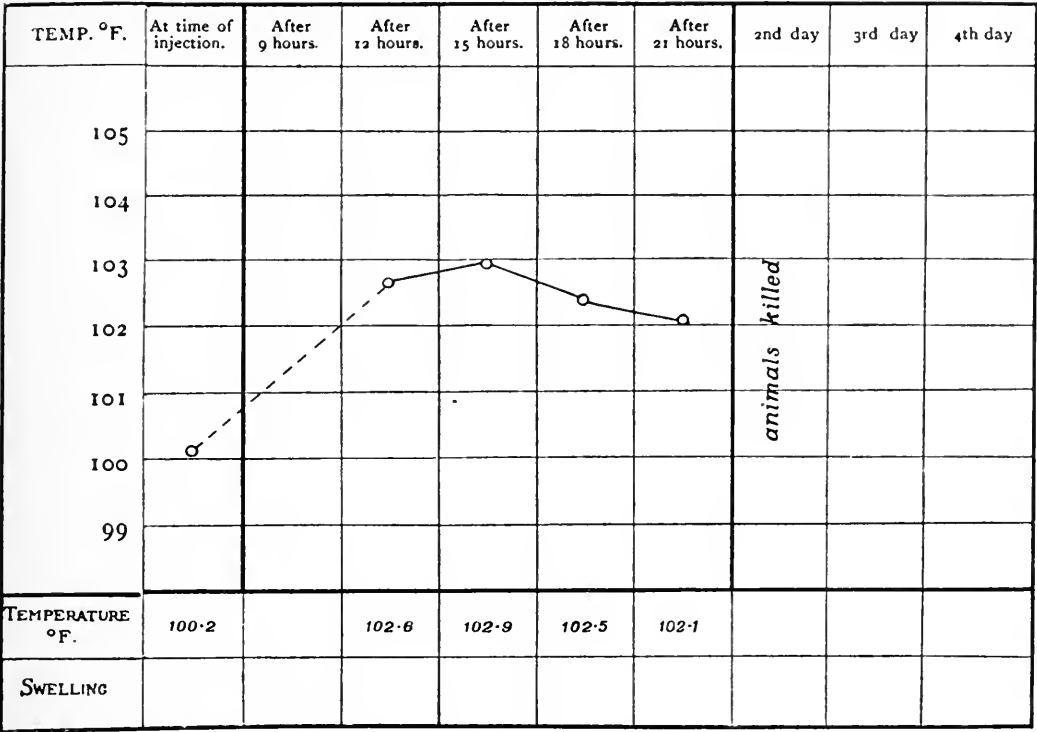


Chart III. Average temperatures of 19 glandered horses which gave large local reactions.

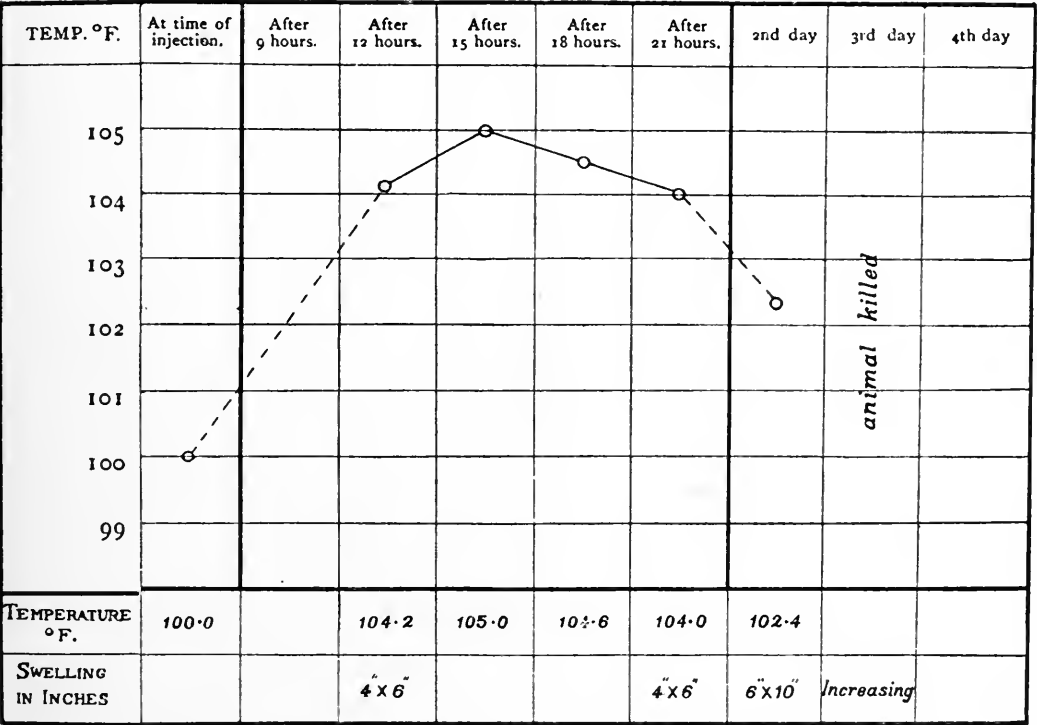


Chart IV. Typical mallein reaction of a glandered horse (No. 52H).

of horse 39K the highest temperature was reached 9—12 hours after injection and the fall in temperature was comparatively rapid. The local swelling did not increase very rapidly but became more diffuse than is the case with most glandered horses.

The temperature reaction in the case of mallein is of great help in diagnosing glanders, but must rank of secondary importance compared with the local swelling. Chart V gives the average temperature of seven horses, tested before admission to the stables, which gave decided local swellings but no large rise in temperature.

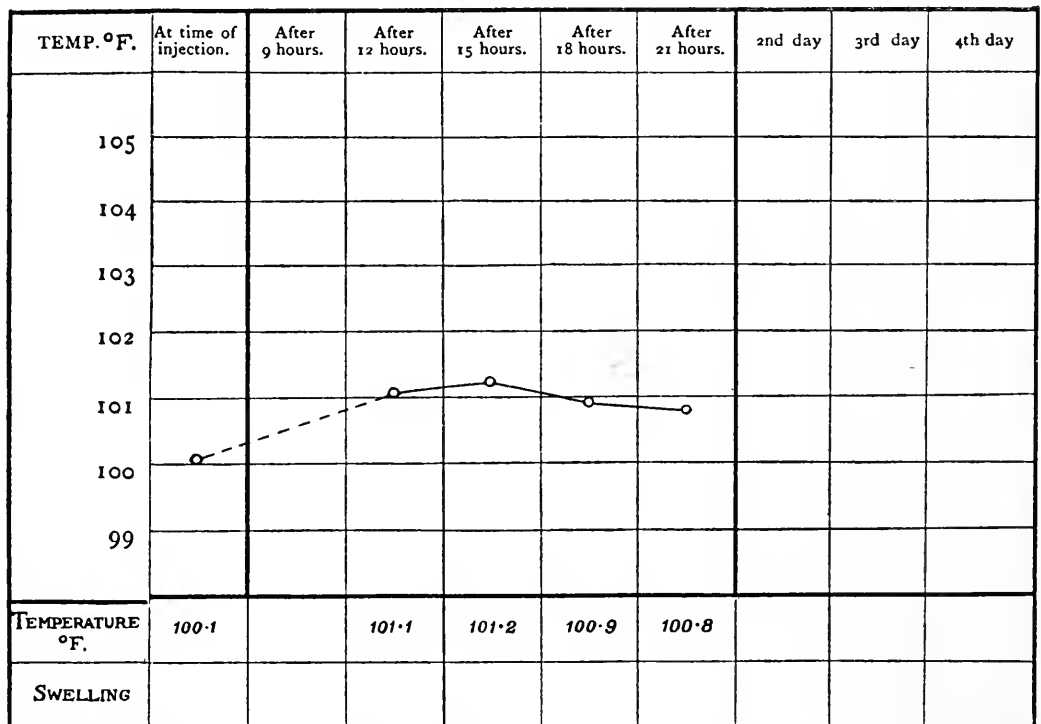


Chart V. Average temperatures from 7 glandered horses which gave large local reactions with very little rise in temperature.

These were condemned as glandered and in all seven cases post-mortem examination showed the correctness of the diagnosis. Although a rise of only one degree was obtained, yet again it is noticed that the highest temperature is after 15 hours and that very little fall takes place in the temperature during the next few hours.

When horse 39K was originally bought it was subjected to the mallein test and passed satisfactorily (see Chart VI).

Since that time the horse had not been exposed to any possibility of infection with glanders.

It was thought that the reaction obtained with horse 39K might

be due to the fact that the horse had been treated with diphtheria toxin for two years. Further experiments were therefore carried out to determine whether a reaction was always obtained upon injecting with mallein horses which had been previously immunised against diphtheria toxin or other bacterial products.

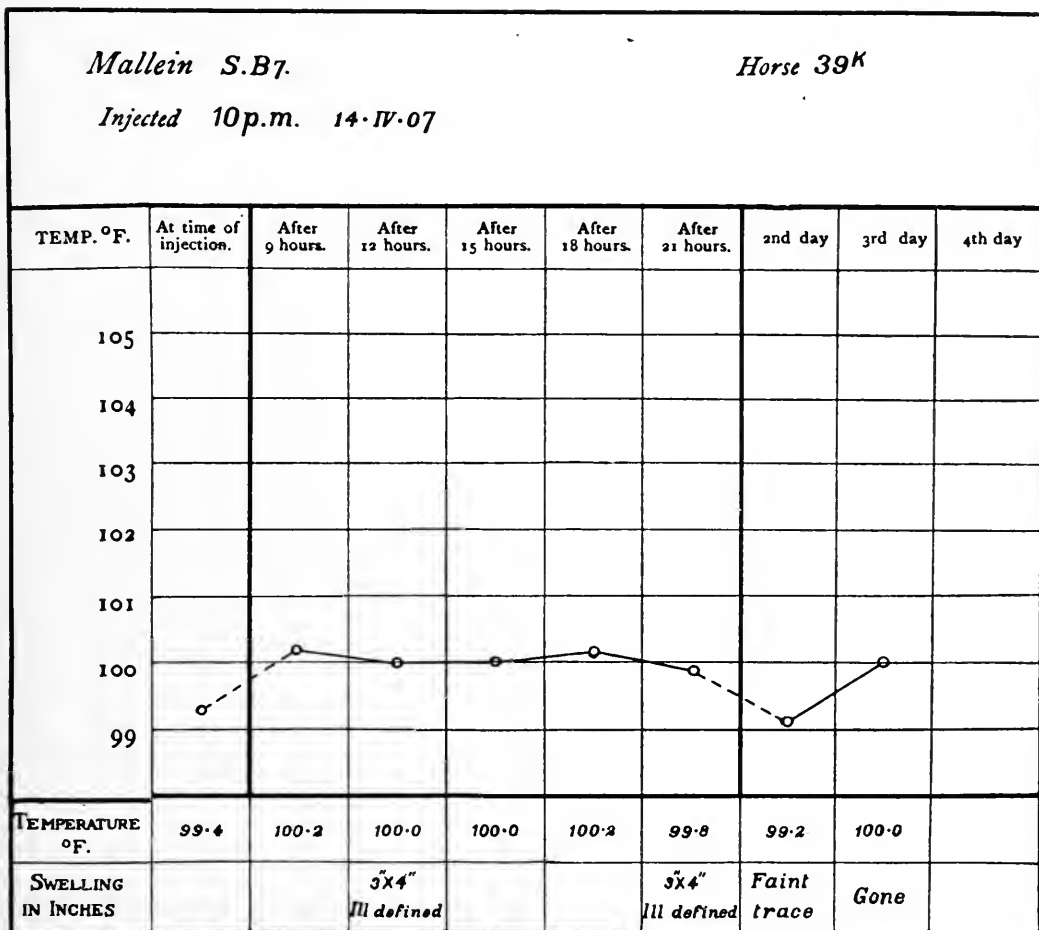


Chart VI. Original mallein reaction of horse 39K.

The Action of Mallein upon Immunised Horses.

The experiment was repeated on a large number of immune horses, with the result that a number of them showed large local swelling but usually no temperature reaction. At the end of twelve hours the local swelling is quite typical of a glanders reaction, but at the end of 24 hours it has commenced to disperse although in some instances it remains large but diffuse until the end of the second day.

Table I gives the results obtained by injecting mallein into 56 horses in various stages of immunisation. The last three columns give the

Non-Specific Mallein Reactions

TABLE I.

Horse	Date of inj.	Swelling ¹			Temperature					No. of mths. of treatment	Details	Days after last inj.
		12 hrs.	36 hrs.	50 hrs.	Time of inj.	12 hrs.	15 hrs.	18 hrs.	21 hrs.	2nd day		
1D	28/5/07	5 × 7 S	7 × 12 I. D.	Faint trace	99.8	99.8	99.8	99.2	100.0	100.0	Strept.	7
2F ²	"	4 × 5 S	I. D.	Gone	101.6	102.0	102.0	101.0	100.4	100.6	Strept.	7
3F	9/6/07	2 × 3 S (L)	F. trace ()	Gone	100.6	100.4	100.6	100.4	101.0	100.6	Diph.	9
4B	2/6/07	2 × 4 F. S.	2 × 3 V. I. D.	F. trace, gone 4th day	100.4	100.0	100.4	101.0	100.6	99.8	Strept.	5
5H	28/5/07	2 × 2 V. I. D.	Gone		99.8	100.4	100.2	100.0	100.0	100.0	Diph. & Strept.	4
6L	"	4 × 5 I. D.	Gone		99.6	99.8	101.0	101.4	101.4	100.0	Meningo	4
7Q	"	3 × 3 I. D.	3 × 3 V. I. D.	Gone	100.2	100.0	100.0	100.4	100.4	100.0	Normal	—
8Q	"	Nil	Gone		100.0	99.8	99.0	99.4	99.0	99.2	Venom	10
9K	29/5/07	1 × 2	Gone		100.0	100.4	100.0	100.2	100.0	99.8	Diph.	9
10E	28/5/07	4 × 6 S	4 × 6 I. D.	Faint trace	99.8	99.8	100.0	100.2	100.0	99.8	Strp., Dip. & Ven.	3
11G	"	2 × 2 V. I. D.	Gone		100.4	99.2	99.6	99.6	99.6	99.6	Diph. & Strept.	4
12M	2/6/07	4 × 4 F. S.	3 × 3 V. I. D.	F. trace, gone 4th day	100.4	100.0	100.4	101.0	100.6	100.0	Typhoid I. V.	5
13E	28/5/07	3 × 4 S	I. D.	Gone	100.2	101.0	101.4	101.0	100.0	99.6	Strept.	7
14B	"	3 × 3 I. D.	Faint trace	Gone	99.8	100.0	101.2	101.2	100.4	99.2	Strept.	7
15R	"	Trace	Gone		100.0	99.6	99.8	100.4	100.4	99.8	Diph.	11
16P	"	3 × 3 I. D.	2 × 4 V. I. D.	Gone	99.8	99.8	99.2	99.0	99.6	99.8	Strept.	7
17C	"	2 × 2 V. I. D.	Gone		100.6	100.0	100.4	100.2	100.4	100.0	Diph.	22
18K	9/6/07	2 × 3 I. D.	Gone		99.8	99.8	99.8	99.8	99.8	100.0	Diph.	10
19G	"	3 × 6 VS (L)	F. trace (L)	F. trace, gone 4th day	99.6	100.0	99.8	99.6	100.2	99.6	Diph.	9
20K	28/5/07	4 × 6 I. D.	Faint trace	Gone	99.6	99.0	99.0	99.0	99.6	99.2	Diph. & Strept.	4
21F	2/6/07	2 × 3 I. D.	Faint trace	Gone	99.6	99.2	99.6	99.6	99.6	99.6	Strept.	5
22G	28/5/07	4 × 5 V. I. D.	Faint trace	Gone	99.8	99.8	99.0	99.0	99.0	99.6	Strept.	4
23M	9/6/07	3 × 5 S (L)	Faint trace	Gone	99.6	99.2	99.0	99.2	99.6	99.2	Diph.	9
24P	2/6/07	3 × 4 I. D.	Faint trace	Gone	100.0	99.8	99.0	99.6	99.8	99.6	Meningo	5
25H	28/5/07	3 × 4 I. D.	3 × 5 I. D.	Faint trace	100.0	99.8	100.0	100.6	100.6	99.8	Diph., Meningo IV	5
25K	9/6/07	2 × 3 V. I. D.	Gone		99.6	99.4	99.6	99.2	99.8	99.8	Normal	—
26K	28/5/07	4 × 4 F. S.	5 × 5 I. D.	Faint trace	100.0	99.6	100.0	100.0	99.8	99.6	Diph.	11
27K	9/6/07	5 × 5 S (L)	Faint trace	Gone	100.0	99.2	99.2	99.0	99.8	99.2	Pneumo IV, Diph.	23
28K	28/5/07	2 × 5 V. I. D.	6 × 6 I. D.	Gone	100.4	102.4	102.0	100.4	99.8	101.2	Diph. & Strept.	1
28K	"	4 × 5 F. S.		Faint trace	100.0	99.6	99.0	99.0	99.2	99.6		4

29M	28/5/07	3 × 3 I. D.	4 × 6 V. I. D.	Faint trace	100.4	100.0	101.0	101.0	100.0	99.6	5	Coli	4
30F	2/6/07	2 × 2 V. I. D.	Faint trace	Gone	100.2	103.6	103.0	101.8	101.0	100.0	39	Diph. & Strept.	5
31F	28/5/07	4 × 5 S (L)	3 × 6 V. I. D.	Gone	100.0	99.6	99.6	100.0	99.8	99.6	27	Diph. & Strept.	5
32M	28/5/07	3 × 5 I. D.	Faint trace	Gone	99.8	99.6	99.6	99.6	99.4	99.6	29	Diph. & Staphy.	4
33N	2/6/07	3 × 5 S (L)	Gone	Gone	99.2	99.6	99.0	99.2	99.2	99.0	1	Melit. IV	5
34K	28/5/07	Faint trace	Gone	Gone	100.4	100.0	99.8	99.8	100.2	99.8	8	Strept.	4
35G	9/6/07	4 × 4 I. D.	Faint trace	Gone	100.2	99.6	99.6	99.6	100.0	99.8	5	Diph.	9
36L	28/5/07	5 × 5 S	6 × 6 V. I. D.	Gone	99.8	100.0	99.8	100.2	100.0	100.0	11	Diph.	11
37G	2/6/07	4 × 6 S (L)	F. trace (L)	F. trace, gone 5th day	99.2	100.0	99.6	99.8	99.6	99.6	15	Typhoid	5
38C	28/5/07	3 × 3 V. I. D.	Gone	Gone	100.6	99.8	100.4	100.0	100.0	99.8	32	Diph.	11
39K	9/6/07	4 × 4 S (L)	4 × 4 V. I. D.	Gone	100.8	99.8	100.0	100.0	100.0	99.6	1	Diph.	1
	12/5/07	2 × 3 S	3 × 5	4 × 8	100.0	102.6	102.4	101.8	101.6	100.6	25	Diph.	9
	15/5/07	4 × 4	Larger		100.0	102.8	—	101.6	101.0	—	36	Diph.	12
40C	9/6/07	4 × 6 S (L)	4 × 4 I. D.	Faint trace	100.4	100.0	99.8	100.0	100.4	99.6	36	Diph.	9
41N	28/5/07	4 × 4 I. D.	Trace	Gone	100.0	99.6	99.2	99.2	99.8	99.8	1	Coli IV	3
42D	2/6/07	3 × 3 I. D.	Faint trace	Gone	100.0	101.0	101.6	101.6	101.8	100.4	8	Typhoid IV	3 mos.
43G	28/5/07	2 × 3 I. D.	Faint trace	Gone	100.2	99.6	99.8	100.0	100.2	99.6	3	Typhoid IV	5
44B	28/5/07	Trace	Gone	Gone	99.8	99.8	100.0	99.8	100.0	99.2	46	Diph. & Strept.	7
45G	2/6/07	3 × 6 V. I. D.	Gone	Gone	99.8	99.6	100.6	100.6	101.0	99.8	0	Normal	—
46E	2/6/07	2 × 3 I. D.	Faint trace	Gone	99.4	99.6	99.8	99.8	99.8	99.2	14	Dysentery	5
47E	9/6/07	4 × 6 S (L)	Faint trace	Gone	100.6	100.0	99.8	100.0	100.2	99.8	14	Staph. & Diph.	9
48F	28/5/07	4 × 6 F. S.	Trace	Gone	100.0	100.6	101.6	101.6	101.6	100.0	15	Diph.	11
49B	2/6/07	3 × 3 F. S.	4 × 5 I. D. (L)	4 × 5 I. D. (L), gone 7th day	99.8	99.6	101.0	100.0	101.0	100.0	7 ^v	Tub. IV. & Diph.	6
50H	9/6/07	2 × 3 I. D.	Gone	Gone	99.6	100.0	100.2	99.8	100.2	99.2	0	Normal	—
51B	28/5/07	3 × 4 I. D.	Gone	Gone	99.6	100.0	99.2	99.6	99.8	99.2	10	Typh. & Diph.	6
52L	28/5/07	3 × 3 V. I. D.	Gone	Gone	99.6	99.2	99.2	99.8	99.8	99.6	2	Diph.	8
53E	2/6/07	4 × 6 S	4 × 6 I. D.	Gone	101.0	99.2	100.0	100.6	100.6	100.0	2	Diph.	8
56B	28/5/07	3 × 6 S (L)	3 × 6 V. I. D.	F. trace, gone 4th day	100.2	100.2	100.6	100.0	100.6	99.6	10	Gono.	5
59B	28/5/07	3 × 5 S	3 × 5 I. D.	Gone	100.0	100.0	100.0	100.0	100.0	99.8	32	Diph.	11

¹ 5 × 7 etc. indicate the size of the local swelling in inches.

No. 2F. This horse was subsequently destroyed for other causes and a very careful examination of the lungs showed no trace of glanders. Swelling is given in inches. S indicates a sharply-defined swelling, F. S. fairly sharp, I. D. ill-defined, V. I. D. very ill-defined. (L) indicates enlarged lymphatics, (T) tender swelling.

number of months of treatment, description of toxin or organism used, and the number of days since the last injection.

It need hardly be added that none of these horses reacted to mallein before treatment, no horse being introduced into the stables unless it has satisfactorily passed the test.

It will be noticed that a large number of these horses gave decided local swellings. An analysis of the results shows that the size of swelling in general depends on the degree to which the horse has been immunised.

In the following tables the reactions are divided as follows:—

Large reaction. Well-defined sharp swellings over 3×3 or ill-defined swellings over 4×4 (inches).

Medium reaction. Any well-defined swelling under 3×3 ; an ill-defined swelling of 4×4 or any swelling not dispersing rapidly.

Small reaction. Any ill-defined swelling under 4×4 dispersing rapidly.

Horses immunised against Diphtheria Toxin.

In Table II the results are tabulated for all horses that have been used solely for the production of diphtheria antitoxin serum.

TABLE II. *Horses injected with diphtheria toxin, grouped according to length of immunisation.*

Number of months under treatment	Large reaction	Medium reaction	Small reaction
1—6	Horse 53	Horse 3	Horse 9
	—	35	17
	—	—	18
	—	—	52
	Total 1	2	4
7—12	Horse 23	—	Horse 15
	36	—	—
	Total 2	0	1
Over 12	Horse 19, 26	—	—
	38, 40	—	—
	47, 48	—	—
	59	—	—
	Total 7	0	0

From this table it will be seen that all horses that have been immunised with diphtheria toxin for over twelve months give large local

reactions with mallein. Of the horses that have been treated for periods of less than six months numbers 9, 17, 18, and 52, which give little or no swelling, are all of very low antitoxic value, while horse 53 with a large swelling has undergone the same treatment, but has yielded comparatively high value serum (600 units per c.c.) for so short a time (2 months). Subsequently (after three months' treatment) this horse yielded serum of 1700 unit value.

Horses 3 and 35 have yielded medium value serum and both give medium reactions. Table III gives the results grouped according to the antitoxic value of the horses.

TABLE III. *Horses injected with diphtheria toxin, grouped according to antitoxic value.*

Antitoxic value	Large reaction	Medium reaction	Small reaction
Under 250 units per c.c.	—	—	Horse 9
	—	—	17
	—	—	18
	—	—	52
Total	0	0	4
250—400	—	—	—
Total	0	0	0
400—600	Horse 26	Horse 3	Horse 15
	38	35	—
	47	—	—
	53	—	—
Total	4	2	1
Over 600	Horse 19	—	—
	23	—	—
	36	—	—
	40	—	—
	48	—	—
	59	—	—
Total	6	0	0

The result obtained with horse 15 seems out of accord with the other results, for although this horse has yielded serum of value between 400 and 600 units per c.c. for the past eight months, yet no reaction was obtained with mallein. Horse 38 gave a similar result at the first injection but subsequently when the injection was repeated it gave a large reaction.

From these results it appears that the size of the mallein swelling is related to the antitoxin producing power of the horse. It is possible that the length of treatment after the horse has once been immunised has little effect upon the swelling and that the results recorded in Table II are due to the fact that no horses are kept for any length of time unless they are yielding high value serum. It is well known that the antitoxin producing capacity of a horse reaches a maximum, and that after varying lengths of time there is a gradual diminution of value until comparatively little antitoxin is produced. Experiments will be carried out to ascertain whether the horses in this condition will react or not to mallein.

Horses immunised against Bacteria and their Products other than Diphtheria Toxin.

These results are not so uniform as those obtained with the horses used for the production of diphtheria antitoxin. In no case has any horse which has been under treatment for less than six months given a large reaction, but several horses failed to react to mallein after over three years' *Streptococcus* treatment. Further experiments are being carried out to ascertain whether, during a prolonged course of treatment, horses, having passed the stage when their reaction (to mallein or other substances) has been at a maximum, finally reach a stage at which their reactivity decreases or even disappears. In the case of diphtheria antitoxin production, this is of regular occurrence, and after varying lengths of time high value horses become comparatively useless. A similar state may be reached in diphtheria horses by overdosing and an example of this occurs amongst the horses used for the production of bactericidal sera. Number 32 once received, by mistake, a large overdose of *Staphylococcus*, after this the animal was useless for the production of antisera. The opsonic index to *Staphylococcus* remained constantly below normal. This horse gave no reaction to mallein.

The general indications are that during the first few months of treatment no mallein swelling is obtained, but that, when once it is well immunised, the animal will react. Also it would appear that after some time, depending partly upon the constitution of the horse and partly upon the nature of its treatment, it ceases to react. Horses under very severe treatment such as *Meningococcus* (No. 24) and *B. dysenteriae* (No. 46) failed to react after over two years' treatment. This indicates a paralysing effect upon the reacting capacity of the animal, but that

the horse is still capable of producing antibodies is shown by the high opsonic index and good clinical results still obtained with the serum of number 24.

The results are not confined to horses treated with subcutaneous injections. Of the horses injected intravenously numbers 12 and 43 form a good contrast; both have been immunised against typhoid endotoxin for the same length of time (3 months) but horse 43 can now tolerate over ten times as much as number 12, and its temperature reaction is usually much lower. On comparing the mallein results of these two horses it is seen that 12 gives a marked reaction and 43 gives none. The protective value of the serum from these two horses does not show any marked difference, and the opsonin, agglutinin and precipitin values closely agree.

In the case of one horse (No. 8) which has been injected with venoms and not with any bacterial products, no mallein reaction was obtained although the horse is capable of producing high value antivenin. The other (No. 10) injected with venoms had been previously treated with bacterial products and now gives a large swelling with mallein.

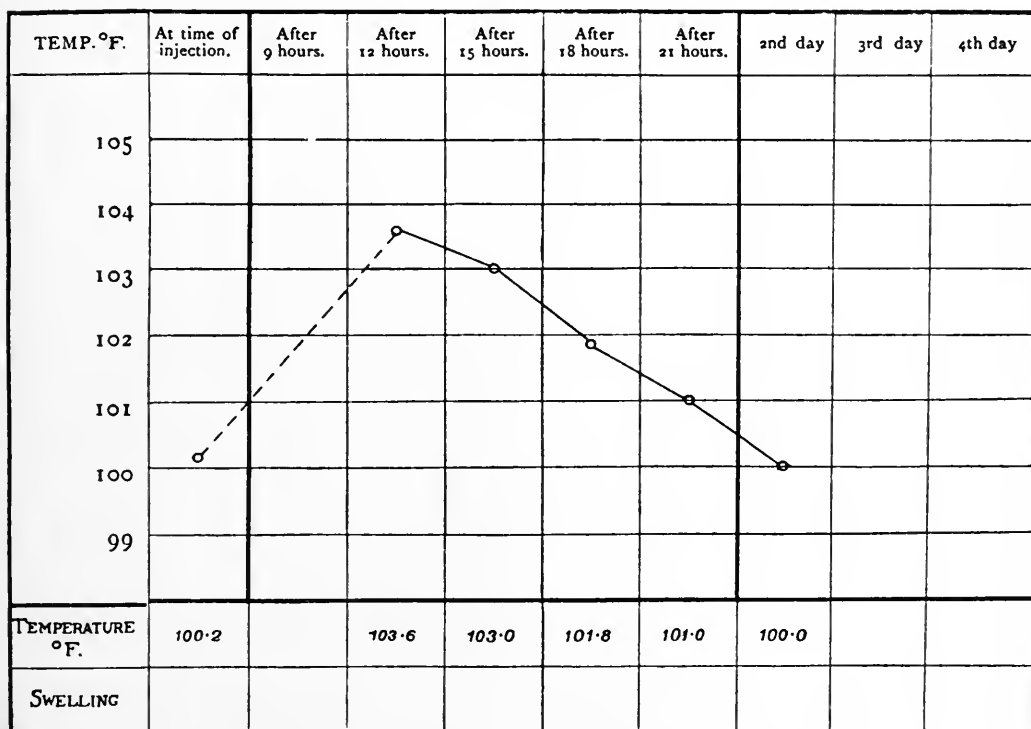


Chart VII. Mallein reaction of horse 30.

The Temperature Reactions.

In only a few instances has there been any marked temperature reaction. In the original case of horse 39K there were both local reaction and rise in temperature; horse 30F (after 39 months of treatment) gives a rise of over 3° F. but no local reaction. In the case of horse 27 the temperature rise may be accounted for by the fact that the horse received the dose of mallein on the day after an injection with diphtheria toxin. The temperature curves of these two horses are plotted in Charts VII (horse 30) and VIII (horse 27).

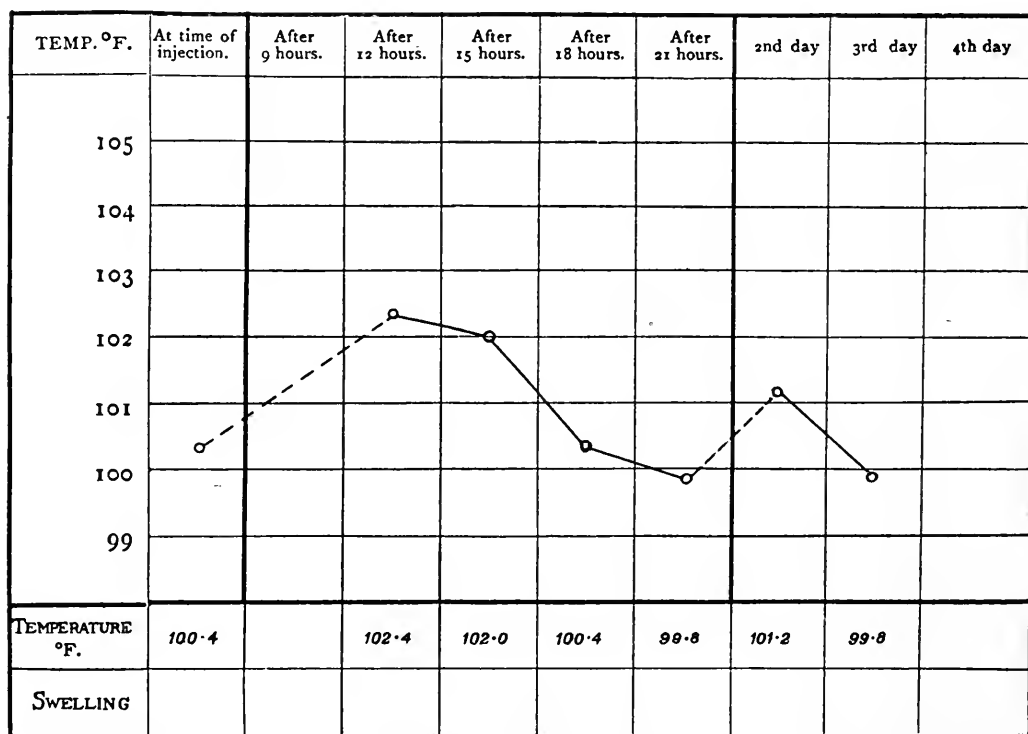


Chart VIII. Mallein reaction of horse 27.

It will be noticed that they resemble the horse 39K curve (Charts I and II) in their rapid fall. The other horses to give a rise of over 1° F. were 13 (*Streptococcus*) and 48 (*B. diphtheriae*), which also gave local reactions, 6 (*Meningococcus*) and 49 (*B. diphtheriae*) with medium swellings, while 14 (after 4 years' *Streptococcus* treatment) and 42 (on intravenous treatment) gave no local reactions.

Action upon Immune Horses of Bacterial Products other than Mallein.

A series of experiments were carried out to ascertain whether other bacterial products injected hypodermically into immune horses cause local reactions similar to those obtained with mallein.

In these experiments the horses were mostly injected on both sides of the neck with different products. This has been our regular proceeding when testing the relative strength of different batches of mallein and we have never found an injection upon one side of the neck to affect the local reaction upon the other side. In these experiments the temperature results cannot be of much importance as any rise in temperature may be due to either or both of the injections. We have noticed that two injections of mallein cause no higher rise than a single dose.

In the following tables the last column records the nature of the injection upon the other side of the neck to that upon which the horse was injected with the product dealt with in the table. Details of the previous treatment of the horses are given in Table I.

Tuberculin.

Table IV gives the results of injecting 13 horses with tuberculin. Large local reactions were obtained with horses 1, 2, 10, 28, 36, 38, all well immunised horses; horse 26 gave nil at first but a large swelling resulted from repeating the dose, and horse 44, a well immunised *Streptococcus* horse, gave only a small reaction. The other horses giving small reactions had been under treatment for only a short time. All horses satisfactorily passed the tuberculin test before admission to the stables.

Coli Products.

In these experiments the filtrate was used from a three months old broth culture of *B. coli*. One-tenth of a c.c. was found sufficient to give decided local reactions with well immunised horses such as Nos. 2, 10, 13, 36, 48, and 59, but only small swellings were obtained with No. 7 (normal), 8 (venom), 45 (normal), and 52 (low value diphtheria horse). Numbers 14 (*Streptococcus*), and 38 (diphtheria), were the only well immunised horses that failed to give a local reaction. Horse 48 (diphtheria) again gave a rise of over 1.0° in temperature (see mallein results, Table I). Horse 29M, which had been treated for the past five months with killed cultures of *B. coli*, gave a fairly sharp reaction, more marked than was the case in the mallein test.

Full details of these results are recorded in Table V.

TABLE IV. *Tuberculin* (2.5 c.c.).

Horse	Date of injection	Swelling		Temperatures					Days after last inj.	Other inj.
		12 hrs.	36 hrs.	Time of inj.						
				12 hrs.	15 hrs.	18 hrs.	21 hrs.	2nd day		
1D	4/6/07	3 × 4 S (L)	4 × 6 S	101.2	100.0	99.8	100.0	100.0	4	—
2F	31/5/07	4 × 6 S (L)	F. trace	100.2	100.6	100.2	100.6	100.0	10	—
7Q	"	3 × 4 I. D.	F. trace	100.4	100.6	100.0	100.2	99.8	—	—
8Q	"	F. trace	Gone	99.8	99.8	99.6	100.2	99.8	13	—
10E	"	4 × 5 I. D.	4 × 5 sharper	99.6	100.0	100.0	100.2	100.0	13	—
25H	4/6/07	F. trace	Gone	100.2	100.0	99.8	100.4	99.8	—	—
26K	"	Nil	—	100.0	99.6	99.2	99.8	99.0	18	—
28K	9/6/07	5 × 5 S (L)	F. trace	100.0	99.2	99.2	99.8	99.2	23	Mallein
34K	4/6/07	3 × 4 F. S.	F. trace	99.8	99.6	99.0	99.0	99.6	4	—
36L	"	Nil	—	100.0	99.2	99.6	99.2	99.2	4	—
38C	31/5/07	5 × 5 S (L)	F. trace	100.0	100.2	100.0	100.0	99.6	14	—
41N	4/6/07	4 × 4 S	F. trace	100.0	100.6	100.4	100.0	99.6	18	—
44B	4/6/07	5 × 5 S (L)	5 × 5 V. I. D.	100.8	99.8	100.0	100.0	99.6	23	Mallein
	4/6/07	3 × 3 I. D.	F. trace	99.8	99.6	99.8	99.6	99.6	4	—
	"	3 × 3 I. D.	Gone	100.4	99.8	100.2	100.0	99.8	4	—

TABLE V. *Coli* (0.1 c.c.).

2F	3/6/07	4 × 5 S (L)	4 × 6 F. S. (L)	F. trace, gone 4th day	100.0	99.8	100.0	100.0	99.8	13
7Q	"	2 × 2 I. D.	2 × 2 I. D.	F. trace, "	99.8	99.6	100.0	100.0	99.8	—
8Q	"	3 × 3 I. D.	5 × 5 V. I. D.	F. trace, "	99.2	99.8	99.8	99.2	99.6	2
10E	"	4 × 6 S (L)	5 × 6 I. D.	F. trace, "	100.0	99.8	100.0	100.0	99.2	2
13E	2/6/07	4 × 4 F. S. (L)	3 × 4 I. D.	Gone	99.6	100.2	99.6	100.6	100.2	12
14B	3/6/07	3 × 3 I. D.	2 × 3 V. I. D.	Gone	100.0	100.0	99.8	99.8	100.6	13
29M	2/5/07	3 × 3 F. S.	3 × 3 F. S.	F. trace, gone 4th day	99.8	99.6	99.6	100.2	99.8	9
36L	3/6/07	3 × 6 F. S. (L)	3 × 8 F. S.	2 × 3 V. I. D., gone 4th day	99.8	100.0	99.8	99.8	99.2	17
38C	"	3 × 3 V. I. D.	2 × 3 V. I. D.	F. trace, gone 4th day	100.2	100.0	100.4	100.0	100.6	17
45G	31/5/07	2 × 3 I. D. (L)	2 × 3 I. D. (L)	Gone	100.0	99.6	100.0	100.6	99.8	—
48F	3/6/07	3 × 6 F. S.	3 × 6 I. D.	F. trace, gone 4th day	99.8	100.0	100.6	101.4	100.2	17
52K	2/6/07	3 × 4 I. D.	F. trace	Gone	99.8	99.6	99.2	99.6	99.2	13
59B	"	3 × 4 S (L)	4 × 4 I. D.	3 × 4 V. I. D., gone 4th day	99.8	99.8	99.2	100.2	100.0	16

TABLE VI. *Typhoid*.

Horse	Date of inj.	Swelling			Temperatures					Days after last inj.	Other inj.
		12 hrs.	36 hrs.	50 hrs.	Time of inj.	12 hrs.	15 hrs.	18 hrs.	21 hrs.	2nd day	
1D	9/6/07	3 × 4 S (L)	2 × 4 V. I. D.	F. trace, gone 5th day	100.0	100.0	101.0	100.4	100.4	100.0	9 Ac. lactici
3F	16/6/07	3 × 3 V. I. D.	3 × 4 V. I. D.	F. trace, gone 4th day	100.6	100.0	100.2	100.0	100.0	100.0	16 Phenol
5H	9/6/07	3 × 3 V. I. D.	F. trace	F. trace, gone 5th day	99.8	99.6	99.6	99.6	99.6	99.8	5 Ac. lactici
6L	"	2 × 2 V. I. D.	Trace	Gone	100.2	100.0	100.2	100.0	100.0	99.6	5 "
13E	"	2 × 3 S (L)	3 × 5 S (L)	5 × 5 F. S. (L), gone 5th day	99.8	99.2	99.6	99.2	99.8	99.2	5 "
14B	"	3 × 3 I. D.	F. trace	Gone	100.2	99.2	100.0	100.2	100.6	99.6	5 "
19G	16/6/07	3 × 4 F. S.	F. trace	F. trace, gone 4th day	99.6	100.0	100.0	100.0	100.2	99.2	16 Phenol
23M	"	2 × 3 I. D.	F. trace	Gone	99.4	99.2	99.0	99.2	99.2	99.2	16 Phenol
48F	20/6/07	3 × 7 S	4 × 5 I. D.	F. trace	99.8	99.6	99.6	99.2	100.0	99.6	16 Diphtheria
52K	4/6/07	2 × 2 I. D.	F. trace	Gone	99.8	99.6	99.2	99.2	99.8	99.6	15 —

TABLE VII. *Enteritidis* (0.5 c.c.).

11G	20/6/07	3 × 3 I. D.	4 × 4 I. D.	F. trace, gone 4th day	99.8	99.8	99.6	99.6	99.6	100.0	6 Staphylococcus
17C	"	3 × 3 I. D.	F. trace	Gone	100.2	99.2	99.2	100.0	100.4	99.8	6 "
29M	"	3 × 4 S	4 × 5 I. D.	Gone	100.0	99.6	99.6	99.0	99.6	99.8	6 "
30F	16/6/07	3 × 3 V. I. D.	3 × 3 V. I. D.	F. trace, gone 4th day	99.6	99.2	99.6	99.6	99.2	99.6	9 Pyocyaneus
31F	"	2 × 4 S (L)	F. trace	F. trace, gone 4th day	100.0	99.2	99.6	99.6	99.8	99.2	9 "
32M	4/6/07	3 × 3 I. D.	3 × 4 V. I. D.	Gone	99.8	99.6	99.6	99.6	99.2	99.0	4 —
38C	20/6/07	2 × 3 V. I. D.	Gone	—	100.0	99.8	99.8	99.6	100.0	99.6	6 Staphylococcus
47E	16/6/07	3 × 4 S	3 × 3 V. I. D.	F. trace, gone 4th day	99.6	99.6	99.6	99.6	99.6	99.2	16 Pyocyaneus
49B	"	3 × 4 I. D.	3 × 5 I. D.	F. trace, gone 4th day	99.6	99.8	100.0	99.8	99.6	99.6	20 "

TABLE VIII. *Streptococcus* (1.0 c.c.).

1D	30/6/07	3 × 3 V. I. D.	F. trace	Gone	100.6	100.6	100.6	100.0	100.0	100.0	6 Proteus
2F	"	2 × 3 V. I. D.	1 × 2 I. D.	Gone	99.8	102.2	101.0	100.0	100.0	99.8	6 "
8Q	"	2 × 4 I. D.	3 × 6 V. I. D.	F. trace, gone 4th day	99.6	100.4	101.0	100.8	100.4	99.6	5 "
10E	"	F. trace	Gone	—	99.6	100.0	99.8	99.8	100.0	99.8	5 "
13E	"	3 × 3 S (L)	3 × 3 S (L)	4 × 6 V. I. D. (L), gone 4th day	100.0	99.6	99.6	100.2	99.8	99.6	6 "
14B	"	F. trace	F. trace	Gone	99.8	99.2	99.2	99.2	99.2	99.2	6 "
59B	"	3 × 4 S	4 × 6 S	4 × 6 I. D.	99.8	99.0	99.2	99.2	99.6	99.8	6 —

Typhoid.

A half c.c. of the filtrate from a three months old broth culture of *B. typhosus* was used.

Large swellings were obtained with horses 1 and 13 (*Streptococcus*), 19 and 48 (diphtheria); all four of these horses had been immunised for a long period. Two well immunised horses, Nos. 14 (*Streptococcus*) and 23 (diphtheria) gave only small reactions. Comparing the swellings caused by the typhoid products with that caused by mallein, only in the case of horse 23 (diphtheria) is there any discrepancy. In no case was there any temperature reaction. Details of results are recorded in Table VI.

Enteritidis.

In Table VII are recorded results obtained by injecting immune horses with 0.5 c.c. of the filtrate from a three months old broth culture of *B. enteritidis*. The only horses to yield well marked reactions were Nos. 31 (*Streptococcus*), 47 (diphtheria), both well immunised horses which had reacted strongly to mallein, and horse 29M (*B. coli*). The reaction of the last horse was more marked after injecting with *B. enteritidis* than with *B. coli*. The well immunised horses failing to react were No. 30, which also gave only a small swelling with mallein, and No. 38, which reacted to mallein but not to *B. coli* products.

Streptococcus.

The results obtained with 1 c.c. filtered broth cultures of *Streptococcus* (of from one to three months' growth) differed somewhat markedly from those obtained with any other bacterial products. Horses 1, 2, and 10 immunised against *Streptococcus* all failed to react, although all these had given decided swellings with mallein and tuberculin, and typhoid or *coli* products; No. 13, the only other *Streptococcus* horse tested, gave a sharp swelling. In the case of horse 2, there was a rise in temperature of 2.4° F., but this horse received 0.5 c.c. proteus products at the same time as the *Streptococcus* injection, so that it is not possible to say which injection caused the temperature reaction. The results are recorded in Table VIII.

Staphylococcus.

One c.c. of the filtrate from a three months old culture of various strains of *Staphylococcus pyogenes aureus* produced swellings similar to those obtained with mallein with one exception; horse 32 (immunised

against *Staphylococcus*) gave no reaction with mallein but responded to the injection of *Staphylococcus* products. The details of the results are given in Table IX.

Diphtheria.

Moderately weak diphtheria toxin was used in doses of 0.5 c.c. The only horses to react were those which had been immunised against diphtheria toxin, numbers 32, 36, and 48; in the case of number 32, no reaction was obtained with mallein. This result is particularly interesting in consideration of the fact that the dose of toxin used together with less than one five-hundredth of a c.c. of the serum from either 36 or 48 would cause no swelling in a guinea-pig. Both horses 10 and 37 which had reacted to mallein failed to react to diphtheria toxin. In no case was there any temperature reaction. The results are recorded in Table X.

Proteus.

Very marked reactions resulted from injecting horses 1 and 13 with 0.5 c.c. of the filtrate from a three months old growth of *B. proteus* on broth. The general results obtained differ considerably from the result of the mallein injection. Horse 8 (venom) gave a reaction while horses 2 (*Streptococcus*) and 36 (diphtheria) failed to do so. It is interesting to note that proteus is the only bacterial product which has given a reaction on horse 8.

A rise in temperature occurred in the case of horse 2 which received an injection of *Streptococcus* products at the same time as the proteus injection. The results are recorded in Table XI.

Pyocyaneus.

Table XII gives the results of injecting 0.2 c.c. of a three months old filtered broth culture of *B. pyocyaneus*. High temperatures were obtained with horse 7, injected at the same time with 1 c.c. of 2.5% phenol; this may be due to the considerable exercise which the horse endured shortly after injecting, and during the following day. Horses 2 (*Streptococcus*) and 47 (diphtheria) were the only animals to produce well-defined swellings. Well immunised animals such as 10, 20, 30, 31, all failed to react.

Acidi Lactici.

The filtrate from a three months' growth on broth of *B. acidi lactici* was used in doses of 0.2 c.c. Table XIII shows that the results do not differ from those obtained after injecting mallein.

TABLE IX. *Staphylococcus* (1.0 c.c.).

Horse	Date of inj.	Swelling		Temperatures							Days after last inj.	Other inj.
		12 hrs.	36 hrs.	50 hrs.	Time of inj.	12 hrs.	15 hrs.	18 hrs.	21 hrs.	2nd day		
8Q	16/6/07	2 × 3 I. D.	F. trace	Gone	99.6	99.2	99.0	99.0	99.0	99.0	1	Diphtheria
10E	"	4 × 5 F. S.	F. trace	Gone	100.0	100.0	99.2	99.0	99.8	99.6	1	"
11G	20/6/07	3 × 3 I. D.	4 × 4 I. D.	Gone	99.8	99.8	99.6	99.6	99.6	100.0	6	Enteritidis
17C	"	F. trace	F. trace	Gone	100.2	99.2	99.2	100.0	100.4	99.8	6	"
29M	"	3 × 4 S	4 × 4 I. D.	F. trace, gone 4th day	100.0	99.6	99.6	99.0	99.6	99.8	6	"
30F	4/6/07	F. trace	Gone	—	100.4	99.2	99.6	99.6	99.6	99.2	7	—
32M	16/6/07	4 × 6 S	F. trace	Gone	99.2	99.6	99.6	99.2	99.2	99.6	5	Diphtheria
37G	"	4 × 5 S (L)	4 × 5 I. D.	F. trace, gone 4th day	99.0	99.2	99.2	99.0	99.0	99.2	10	"
38C	20/6/07	F. trace	Gone	—	100.0	99.8	99.8	99.6	100.0	99.6	6	Enteritidis
45K	4/6/07	1 × 2 V. I. D.	F. trace	Gone	100.2	99.8	99.6	99.6	99.8	99.2	—	—

TABLE X. *Diphtheria* (0.5 c.c.).

8Q	16/6/07	Nil	—	—	99.6	99.2	99.0	99.0	99.0	99.0	1	Staphylococcus
10E	"	3 × 4 I. D.	F. trace	Gone	100.0	100.0	99.2	99.0	99.8	99.6	1	"
32M	"	3 × 6 F. S.	F. trace	Gone	99.2	99.6	99.6	99.2	99.2	99.6	5	"
36L	20/6/07	4 × 5 S	4 × 6 I. D.	3 × 6 V. I. D., gone 4th day	99.8	100.0	100.0	99.8	99.8	99.8	6	Proteus
37G	16/6/07	3 × 4 V. I. D.	3 × 4 V. I. D.	Gone	99.0	99.2	99.2	99.0	99.0	99.2	10	Staphylococcus
48F	20/6/07	2 × 4 F. S.	3 × 4 S (L)	3 × 4 I. D. (L)	99.8	99.6	99.6	99.2	100.0	99.6	6	Typhoid

TABLE XI. *Proteus* (0.5 c.c.).

1D	20/6/07	4 × 6 S (L)	5 × 9 F. S. (L)	F. trace, gone 4th day	100.6	100.6	100.6	100.0	100.0	100.0	6	Streptococcus
2F	"	3 × 3 I. D.	3 × 4 I. D. (L)	Gone	99.8	102.2	101.0	100.0	100.0	99.8	6	"
4B	16/6/07	2 × 3 S (L)	2 × 3 I. D. (L)	2 × 3 V. I. D., gone 5th day	99.6	100.6	100.2	99.8	100.2	99.8	9	—
8Q	20/6/07	3 × 3 S (L)	4 × 6 S (L)	3 × 4 I. D. (L), gone 4th day	99.6	100.4	101.0	100.8	100.4	99.6	5	Streptococcus
9K	9/6/07	2 × 3 I. D. (L)	3 × 4 V. I. D.	Gone	101.0	102.0	101.2	101.2	101.4	—	20	—
10E	"	3 × 4 F. S.	4 × 5 I. D.	Gone	99.6	100.0	99.8	99.8	100.0	99.8	5	Streptococcus
13E	"	4 × 4 S (L)	6 × 6 S (L)	3 × 6 V. I. D. (L), gone 4th day	100.0	99.6	99.8	100.2	99.8	99.6	6	"
14B	"	2 × 2 V. I. D.	F. trace	Gone	99.8	99.2	99.2	99.2	99.2	99.2	6	"
15R	"	3 × 6 I. D.	F. trace	Gone	100.0	99.6	100.0	100.0	100.2	100.0	6	Mallein
16P	16/6/07	3 × 3 S (L)	4 × 4 I. D. (L)	F. trace (L), gone 4th day	100.0	100.0	100.6	100.2	100.0	99.6	5	—
28K	"	3 × 3 I. D.	3 × 3 I. D.	F. trace, gone 4th day	99.2	99.2	99.0	99.2	99.2	99.0	5	—
36L	20/6/07	3 × 4 V. I. D.	3 × 4 V. I. D.	Gone	99.8	100.0	100.0	99.8	99.8	99.8	6	Diphtheria
44B	16/6/07	3 × 3 I. D.	3 × 3 V. I. D.	F. trace, gone 4th day	99.4	99.6	99.6	99.2	99.6	99.2	3	—

TABLE XII. *Pyocyaneus* (0.2 c.c.).

Horse	Date of inj.	Swelling		Temperatures					Days after last inj.	Other inj.
		12 hrs.	36 hrs.	50 hrs.	Time of inj.	12 hrs.	15 hrs.	18 hrs.	21 hrs.	2nd day
2F	9/6/07	3 × 4 S (L)	F. trace	Gone	100.0	99.8	99.8	99.6	99.8	99.6
7Q	"	2 × 3 V. I. D.	F. trace	Gone	99.4	103.2	103.2	102.8	102.4	101.2
8Q	"	2 × 3 I. D.	F. trace	Gone	100.4	99.2	99.2	99.0	99.0	99.2
10E	"	3 × 3 V. I. D.	Gone	—	100.6	100.4	100.2	100.0	100.2	99.8
20K	4/6/07	4 × 5 V. I. D. (L)	F. trace	Gone	99.6	99.2	99.2	99.0	99.2	99.0
30F	16/6/07	1 × 2 I. D.	1 × 2 V. I. D.	Gone	99.6	99.2	99.6	99.6	99.2	99.6
31F	"	2 × 3 I. D.	Gone	—	100.0	99.2	99.6	99.6	99.8	99.2
47E	"	3 × 3 F. S.	4 × 6 I. D.	4 × 4 V. I. D., gone 4th day	99.6	99.6	99.6	99.6	99.6	99.2
49B	"	2 × 3 I. D. (L)	2 × 4 V. I. D.	F. trace	99.6	99.8	100.0	99.8	99.6	99.6

TABLE XIII. *Acid Lactici* (0.2 c.c.).

1D	9/6/07	5 × 7 V. S (L)	6 × 10 S (L)	8 × 14 I. D., gone 5th day	100.0	100.0	101.0	100.4	100.4	100.0	9	Typhoid
5H	"	4 × 4 I. D.	F. trace	Gone	99.8	99.6	99.6	99.6	99.6	99.8	5	"
6L	"	4 × 4 I. D.	F. trace	Gone	100.2	100.0	100.2	100.0	100.0	99.6	5	"
13E	"	4 × 6 S (L)	3 × 5 I. D.	F. trace, gone 4th day	99.8	99.2	99.6	99.2	99.8	99.2	5	"
14B	"	4 × 5 I. D.	Gone	—	100.2	99.2	100.0	100.2	100.6	99.6	5	"
22G	4/6/07	3 × 5 I. D.	F. trace	Gone	99.2	99.6	99.6	99.6	99.2	99.2	4	"
35G	14/6/07	2 × 3 I. D.	3 × 4 V. I. D.	F. trace, gone 4th day	100.0	99.8	99.6	99.6	100.0	99.6	16	Phenol
40C	"	3 × 4 F. S.	Gone	—	99.8	100.0	99.8	99.8	99.8	99.2	16	"

TABLE XIV. *Phenol* (1 c.c. 2.5 %).

2F	9/6/07	2 × 3 F. S.	F. trace	Gone	100.0	99.8	99.8	99.6	99.8	99.6	5	Pyocyaneus
3F	16/6/07	2 × 3 V. I. D.	Gone	—	100.6	100.0	100.2	100.0	100.0	100.0	16	Typhoid
7Q	9/6/07	F. trace	Gone	—	99.4	103.2	103.2	102.8	102.4	101.2	—	Pyocyaneus
8Q	"	2 × 3 I. D.	F. trace	Gone	100.4	99.2	99.2	99.0	99.0	99.2	1	"
10E	"	2 × 2 V. I. D.	F. trace	Gone	100.6	100.4	100.2	100.0	100.2	99.8	1	"
19G	16/6/07	F. trace	2 × 4 V. I. D.	Gone	99.6	100.0	100.0	100.0	100.2	99.2	16	Typhoid
23M	"	1 × 2 I. D.	F. trace	Gone	99.4	99.2	99.0	99.2	99.2	99.2	16	"
35G	"	2 × 2 I. D.	F. trace	F. trace, gone 4th day	100.0	99.8	99.6	99.6	100.0	99.6	16	Ac. Lactici
40C	"	Nil	—	—	99.8	100.0	99.8	99.8	99.8	99.2	16	"
59B	4/6/07	2 × 3 F. S.	2 × 3 F. S.	F. trace, gone 5th day	99.4	99.4	99.2	99.2	99.6	99.2	18	—

Other substances.

The effect of an irritant other than a bacterial product was next tested upon normal and immune horses. One c.c. of 2·5 % phenol was used and the results are recorded in Table XIV. Two horses alone (Nos. 2 and 59) gave only moderate swellings, while well immunised horses such as 10, 19, 23, and 40 gave no reaction. Apparently irritants do not act in the same way as bacterial products; slight reactions may sometimes occur, due to the susceptibility of certain horses to particular substances.

Temperature Reaction.

In two cases only after injection of other bacterial products than mallein was there any marked rise in temperature: horse 2 injected 30/5/07 with proteus and *Streptococcus* products, and horse 7 injected 9/6/07 with *Pyocyaneus* and phenol. The curves in these two cases are similar to those obtained with mallein upon horses 27, 30, and 39.

Conclusion.

Bacterial products act similarly to mallein in giving large local reactions upon certain immune horses. As a general rule, the products from different organisms act similarly, but there are often exceptions. Table XV gives a summary of the results obtained. It will be seen that the horses that reacted to mallein react to the majority of the other products; a few horses like No. 10 are inconsistent in their reactions, but others like Nos. 13 and 14 are quite uniform.

In our experiments no connection can be traced between the reaction and the interval of time elapsing after the last injection received by the horse in the course of its immunisation, nor do the previous small test injections of other bacterial products appear to affect the subsequent reaction.

Effect of repeated doses of mallein.

An opportunity arose of testing the action of repeated doses of mallein upon a glandered horse. The condition of the animal was recognised by the mallein reaction only, no clinical signs of glanders being present. This horse was kept carefully isolated while receiving doses of mallein every four to seven days. The opportunity was also seized of finally injecting a dose of tuberculin with the result that a large reaction was obtained. Upon post-mortem examination the lungs of the horse were found to contain only a few small nodules. It would

TABLE XV.

No.	Mallein	Tuber- culin	Coli	Ty- phoid	Enteri- tidis	Strepto- coccus	Staphy- lococcus	Diph- theria	Proteus	Pyocy- aneus	Acidi- lactici	Phe- nol
1	L	L		L		S			L		L	
2	L	L	L			S			S	L		M
3	M			S								S
4	M								M			
5	S			S							S	
6	M			S							S	
7	S	S	S							S		S
8	S	S	S			S	S	S	M	S		S
9	S								S			
10	S	L	L			S	L	S	M	S		S
11	S				S		S					
12	L											
13	L		L	L		L			L		L	
14	S		S	S		S			S		S	
15	S								S			
16	S								M			
17	S				S		S					
18	S											
19	L			L								S
20	M									M		
21	S											
22	M										S	
23	L			S								S
24	S											
25	S	S										
26	L	L										
27	S											
28	L								S			
29	M		M		L		M					
30	S				S		S			S		
31	L				M					S		
32	S				S		L	M				
33	M											
34	S	S										
35	M										S	S
36	L	L	L					L	S			
37	L						L	S				
38	L	L			S		S					
40	L										M	S
41	M	S										
42	S											
43	S											
44	S	S										
45	S		S				S					
46	S											
47	L				L					L		
48	L		L	L				M				
49	M				S					S		
50	S											
51	S											
52	S		S	S								
53	L											
56	L											
59	L		L			L						M

L=large swelling: M=medium swelling: S=small swelling.

be interesting to conduct further researches upon the action of various bacterial products on a number of glandered horses, but unfortunately for obvious reasons this is practically impossible.

At the same time a number of normal horses were injected with mallein every four to seven days.

The general indications were:

1. A glandered horse can be injected within a few days after the first injection and still give a reaction. The dose need not be increased.
2. Healthy horses give increasing reactions upon repeating the dose at short intervals up to about the 4th—5th injection, but these reactions simulate those obtained upon immune horses, rather than those upon glandered animals.
3. After the fifth or sixth injection the reaction again becomes small, but does not entirely disappear.
4. No temperature reaction was obtained either in the glandered horse or in the non-glandered ones after the second dose of mallein.

Summary.

1. Many horses immunised against other bacteria or bacterial products will give a large local reaction to mallein, but this is usually not associated with a rise in temperature. In the case of horses injected with diphtheria toxin, the size of the swelling appears to be related to the degree of immunity attained. This point has not yet been established in the case of horses immunised against other bacterial products.
2. This local reaction disappears rapidly and can thus be distinguished from the reaction in the case of glandered horses.
3. In the few cases when a rise in temperature took place (3 cases out of 56) the curve was markedly different from that obtained in the case of glandered animals.
4. Other bacterial products react similarly to mallein upon immune horses.

EXPERIMENTS ON THE SURVIVAL OF *B. TYPHOSUS* IN STERILISED AND UNSTERILISED SOIL.

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It was found in a series of bacteriological examinations of soil from the grounds of Queen's College, Belfast, that the ordinary saprophytic organisms present do not grow on the Drigalski-Conradi medium. Samples of soil weighing about one gramme were added to flasks containing a fluid medium of the same composition, but without the agar. When incubated at 37° C. some growth of putrefactive bacteria took place, but on plating a few loopfuls from the flask on the corresponding agar medium and again incubating for 24 hours colonies were very rarely found. No Coli-like colonies ever appeared, and the few typhoid-like colonies which were occasionally found proved on further examination to be cocci, frequently *Micrococcus tetragenus*. On the other hand, if a loopful of *B. coli* or *B. typhosus* were added to the flask along with the soil the plates made after incubation showed numerous colonies of these bacilli.

In view of these results it appeared to be worth while to investigate the survival of the typhoid bacillus in soil with the aid of the Drigalski medium.

The soil used was taken at a depth of 3 to 4 inches from under grass in the College grounds. In the first set of observations the soil was placed in wide necked glass stoppered bottles; of these some were sterilised by steam in the autoclave for half-an-hour at 120° C., others were left unsterilised.

Pure cultures of *B. typhosus* and *B. coli* were isolated from the faeces of a typhoid patient on Nov. 17th, 1904, and these were used to inoculate

the soils in the following manner. Surface agar cultures made in Petri dishes and incubated at 37° C. for 24 hours were emulsified in a few cubic centimetres of sterile water and sprinkled as evenly as possible over the surface of the soil, one plate culture being used for each sample. Thus no nutrient material was added to the soil along with the bacilli, a point of importance which has been overlooked in some previous investigations. The bottles were covered with brown paper to exclude the light; some were kept at a constant temperature of 20° C., others were exposed to the natural outside temperatures. Enumerations of the bacilli were made from time to time in the following manner. A small quantity—approximately 1 gramme—of the soil was removed from the surface of the sample with a sterile platinum spud and transferred to a flask containing 100 c.c. sterile water. The flask was weighed before and after the addition of the soil and the amount taken thus ascertained.

The flask was thoroughly shaken and further dilutions of 1 in 100, 1 in 1000, etc. made by transferring 1 c.c. from the flask to a tube containing 9 c.c. sterile water, 1 c.c. of this to another similar tube and so on.

By means of a capillary pipette, delivering 30 drops to 1 c.c., 1/30 c.c. or 1/15 c.c. of the necessary dilutions was added to the surface of Drigalski plates and spread by means of a sterile glass rod bent at right angles. The plates were counted after 24 hours incubation and from the number of colonies appearing a sufficiently approximate estimate of the number of bacilli in 1 gramme of soil could be made. As the number of bacilli decreased the method of isolation indicated in the first paragraph was used. When typhoid-like colonies appeared these were subcultured and tested on all the usual media and by agglutination.

Only a summary of the final results of the first set of experiments is given here. The enumerations do not differ materially from those of the later experiments which are given in full.

Experiments started November 28th, 1904.

Unsterilised soil inoculated with emulsion of *B. typhosus*, moisture 35 %.

	Temperature	<i>B. typhosus</i> present	Absent
Exp. (1)	Outside	After 50 days	In 61 days
„ (2)	20° C.	„ 42 „	„ 50 „

Sterilised soil inoculated with emulsion of *B. typhosus*, moisture 34 %.

	Temperature	<i>B. typhosus</i> present	Absent
Exp. (3)	Outside	After ? days	In 11 days
„ (4)	20° C.	„ ? „	„ 11 „

Unsterilised soil inoculated with emulsions of *B. coli* and *B. typhosus*, moisture 35 %.

Exp. (5) Outside. *B. coli* present in large numbers after 42 days.
B. typhosus not isolated.

Exp. (6) 20° C. *B. coli* present in large numbers after 50 days.
B. typhosus not isolated.

Sterilised soil inoculated with emulsions of *B. coli* and *B. typhosus*, moisture 34 %.

Exp. (7) Outside. *B. coli* present after 13 days, absent in 21 days.
B. typhosus not isolated.

Exp. (8) At 20° C. *B. coli* present after 50 days.
B. typhosus present after 9 days, not found later.

In all the experiments the typhoid bacilli decreased in numbers fairly rapidly, but as will be seen from the above tables, they survived five times as long in the natural soil as in that which had been subjected to sterilisation. This result was contrary to all expectation. In the soils inoculated with a mixture of *B. coli* and *B. typhosus* no conclusions can be drawn with regard to the latter. The results for *B. coli* are not uniform. In the natural soils the bacillus was present in large numbers at the end of 50 days and was showing no signs of dying out. In one of the sterilised samples it had died out in 21 days. In the other, which was kept at a higher temperature, it at first showed a rapid decrease in numbers but later apparently it managed to establish itself. We may conclude that for the *B. coli* also this soil, when sterilised, forms a less favourable medium than it does in its natural condition.

To confirm these results a new set of experiments was started on March 11th, 1905. The moisture present in the soils of the first series (34—35 %) is higher than the average soil moisture during the winter months, which was 27.4 % in 15 samples taken between October and January. The excessive moisture, together with the fact that the samples were kept in stoppered bottles, led to the growth of moulds in some of the samples.

In the next experiments therefore the conditions were altered so as to secure a lower percentage of moisture and better aëration. The soil used was taken from the same place as before.

Different portions of this were treated as follows:—(1) Part was dried at a temperature of from 30° to 40° C. in a current of air. (2) Part was sterilised in the autoclave at 115° C. for 20 minutes and then dried in the hot air oven at 100° C. (3) Part was dried in the hot air oven at 90° to 100° C. without previous sterilisation. Each portion was then powdered in a mortar and 120 grammes placed in the bottom of a wide glass jar with a loosely fitting cover. The depth of earth in

each jar was about 1 inch. The emulsions of typhoid bacilli, made from agar plates as before, were added in the amount of 40 c.c. to each jar and a moisture of approximately 25 % was thus obtained.

The emulsions were made with sterile tap water except in one case where sewage sterilised by filtration through porcelain was used instead. Observations were made with *B. typhosus* only and the same strain was used as in the previous experiments. The jars were covered with brown paper to exclude light and all were kept at outside temperatures.

The enumerations and the isolation of the typhoid bacillus were carried out as before. The samples for examination were taken from the surface except where otherwise stated.

Experiment No. 9. Unsterilised soil dried at 30°—40° C. inoculated with emulsion of *B. typhosus*, March 11th, 1905.

Date	Days	<i>B. typhosus</i> per gramme	Moisture
Mar. 11		84,000,000 (at least)	25 %
„ 14	3	132,000,000	
„ 17	6	156,000,000	
„ 20	9	52,200,000 (at least)	
„ 24	13	1,200,000	
„ 28	17	255,000 (deep sample)	
„ 31	20	9,000,000 (at least)	25 %
Apr. 3	23	540,000 (deep sample)	
„ 5	25	less than 3,000 (none found)	
„ 10	30	„ „ 600 „ „	
„ 11	31	present in $\frac{1}{2}$ gm.	
„ 26	46	present in 1 „	
May 13	63	absent in 1 „	
„ 21	71	present in 1 „	
June 1	82	absent in 1 gm. (Also on June 5 and 12.)	

Experiment No. 10. Sterilised soil dried at 100° C. inoculated with emulsion of *B. typhosus*, March 11th, 1905.

Date	Days	<i>B. typhosus</i> per gramme	Moisture
Mar. 11		152,000,000	26 %
„ 14	3	28,500,000	
„ 17	6	less than 300,000 (none found)	
„ 18	7	1,500	
„ 20	9	100	
„ 22	11	absent in 1 gm.	
„ 24	13	absent in 1 „ (Also on May 13th.)	

Experiment No. 11. Unsterilised soil dried at 30°—40° C. inoculated with emulsion of *B. typhosus* in sterile sewage, March 11th, 1905.

Date	Days	<i>B. typhosus</i> per gramme	Moisture
Mar. 11		124,000,000	23 %
„ 14	3	144,000,000	
„ 17	6	180,000,000	
„ 20	9	102,600,000	
„ 24	13	2,400,000 (deep sample)	
„ 28	17	4,500,000 „ „	
„ 31	20	at least 18,000,000	20 %
Apr. 3	23	300,000 (deep sample)	
„ 5	25	219,000	
„ 10	30	1,800	
„ 26	46	present in 1 gram.	
May 13	63	present in 1 „	
„ 21	71	present in 1 „	
June 1	82	absent in 1 „	
„ 5	86	present in 1 „	
„ 12	93	absent in 1 gram. (Also on June 19th.)	

Experiment No. 12. Soil dried at 90°—100° C., bacteria present after drying at least 24,000 per gramme, inoculated with emulsion of *B. typhosus*, March 23rd, 1905.

Date	Days	<i>B. typhosus</i> per gramme	Moisture
Mar. 23		180,000,000	24.6 %
„ 28	5	36,000,000	
„ 31	8	at least 150,000,000	23 %
Apr. 3	11	990,000 (deep sample)	
„ 5	13	3,000,000	
„ 10	18	16,000	
„ 26	34	present in 1 gram.	
May 13	51	present in 1 „	
„ 21	59	present in 1 „	
June 1	70	absent in 1 „	
„ 5	74	present in 1 „	
„ 12	81	absent in 1 gram. (Also on June 19th.)	

In the sterilised soil (Exp. No. 10) the typhoid bacillus disappeared in 11 days just as in the previous experiments (Nos. 3 and 4), while in the unsterilised soils it survived for periods of 71, 74, and 86 days respectively. The addition of sewage in Exp. No. 11 had little effect in prolonging its survival. Even in the unsterilised soils there is a progressive decrease in the number of the bacilli, this being most rapid after about the 20th day.

The remarkable rapidity with which the typhoid bacillus dies out in this soil when it has been subjected to sterilisation by steam under pressure calls for some explanation. Exp. No. 12 shows that the effect is not produced by dry heat at 100° C. It appeared most probable that

the effect is due to the production during sterilisation of substances which have a bactericidal action, as for example the formation of acids by hydrolysis. No change, however, in the reaction of the soil washings to phenol-phthalein could be made out, and it seemed possible that the presence of the soil bacteria might have a favourable action on the survival of the typhoid bacillus.

To test this question a new series of experiments was started on October 28th. The soil was taken from a different part of the College grounds also under grass. It differed in appearance from that used in the experiments already described, being redder in colour and more sandy. One portion was dried at 80° C. for two or three hours, another was sterilised for 20 minutes at 115° C. in the autoclave and then dried at 80° C. In each case 150 grm. of the dried soil was placed in the glass jar and 50 c.c. of typhoid emulsion distributed as evenly as possible over the surface as in the former experiments.

One sample of the sterilised soil received along with the typhoid bacilli emulsions made from cultures of bacilli isolated from the soil.

Two cultures (*A* and *B*) of bacilli belonging to the "subtilis" group were used. *A* shows a spreading filamentous growth on agar, *B* a tough white growth with little tendency to spread. Pieces of the growths were ground up in an agate mortar with a little water, after dilution the emulsion was centrifugalised to get rid of the larger masses of growth and 5 c.c. were added to the 50 c.c. of typhoid emulsion. The same culture of *B. typhosus* was used and the technique was in every respect the same as before. In addition, enumerations of the spores present were made on gelatin plates, a temperature of 80° C. for 10 minutes being used to kill off non-sporing forms. After the typhoid bacilli had died out counts of the total bacteria growing on gelatin were made.

Experiment No. 13. Soil dried at 80° C. inoculated with emulsion of *B. typhosus*, Oct. 28th, 1905.

Date	Day	<i>B. typhosus</i> per gramme	Spores per gramme	Moisture
Oct. 29	1	250,000,000	8,200	26.7 %
Nov. 3	6	8,000,000		
„ 7	10	25,000,000	6,000	25.1 %
„ 14	17	1,400,000		25 %
„ 23	26	less than 2,000	at least 100,000	
„ 28	31	12,000		
Dec. 7	40	less than 200	400,000	
Jan. 5	69	present in 1 grm.		25.1 %
„ 19	83	absent in 1 grm. (Total on gelatin)	3,320,000 20,000,000	

Experiment No. 14. Sterilised soil inoculated with emulsion of *B. typhosus*, Oct. 28th, 1905.

Date	Day	<i>B. typhosus</i> per gramme	Moisture
Oct. 29	1	80,000,000	27.1 %
Nov. 3	6	8,000,000	
„ 7	10	20,000,000	28 %
„ 14	17	10,000,000	22.4 %
„ 23	26	60,000	
Dec. 7	40	600	
Jan. 5	69	present in 1 gram.	20.4 %
„ 19	83	absent in 1 gram.	

Experiment No. 15. Sterilised soil inoculated with emulsions of *B. typhosus* and soil bacteria, Oct. 28th, 1905.

Date	Day	<i>B. typhosus</i> per gramme	Spores per gramme	Moisture
Oct. 29	1	250,000,000	less than 100	29.2 %
Nov. 3	6	1,800,000		
„ 7	10	12,000,000	12,500	28.5 %
„ 14	17	4,000,000	8,000	
„ 23	26	30,000	5,000	
Dec. 7	40	400	60,000	
Jan. 8	72	present in 1 gram.		23.9 %
„ 19	83	absent in 1 gram.	142,000	
		(Total on gelatin)	11,200,000	

In this series of experiments the typhoid bacillus died out at exactly the same rate in all three samples, the time of survival corresponding to that of the unsterilised soils of the previous series. In this case therefore sterilisation of the soil had no inimical action on the survival of the bacillus, a marked contrast to the results of Exps. Nos. 3, 4, and 10. The addition of soil bacteria to the sterilised soil had no effect on the survival of the typhoid bacillus.

The difference in the behaviour of the sterilised soil in Exp. No. 14 can only be explained by differences in its chemical composition. It appears therefore that in some samples of soil, but not in all, substances are produced during sterilisation which have a bactericidal effect on the typhoid bacillus. The matter requires further investigation.

The progressive increase in the natural soil bacteria in Exps. Nos. 13 and 15, corresponding with the decrease in the typhoid bacilli, is interesting and shows that the soil bacteria are able to utilise the dead typhoid bacilli as food supply. The numbers reached at the close of the experiments are far in excess of those ordinarily present in uncontaminated soil and indicate the presence of organic material suitable for the growth of saprophytes. Active growth of the soil organisms is also shown by the high proportion of vegetative forms in the last counts.

That the soil organisms have not, as has been supposed, an antagonistic action towards the typhoid bacillus is shown by the fact that the latter survived as long in Exps. Nos. 13 and 15 as in No. 14.

Sidney Martin (1897—1900) in an elaborate investigation extending over a number of years, found that in sterilised virgin soils the typhoid bacillus died out rapidly, while in sterilised contaminated soils growth occurred and the bacillus could be recovered after 400 days. His inoculations were made as a rule with broth cultures. In the few experiments where the bacillus was added to the soil without nutrient broth the evidence of growth is not very conclusive. Where nutrient broth is added to the soil even in small amounts the conclusion that the soil *per se* forms a suitable medium for the growth of the bacillus is not warranted; all that can be claimed is that the soil in question has no inimical action on the bacillus. The destructive action of the sterilised virgin soil on the typhoid bacillus even when added in broth culture was probably due, as my results show, to the production of bactericidal substances during sterilisation. This soil was not examined in the natural condition. Contaminated soils, proved when sterilised and inoculated with broth cultures to be capable of supporting the life of the bacillus, were examined without previous sterilisation. When inoculated with broth cultures of *B. typhosus* and incubated at 37° C., in five experiments, the bacillus was recovered once after 50 days, once after 24 hours, and in the remainder not at all. When the samples were kept at lower temperatures the typhoid bacillus could be recovered more easily but never after 12 days. Martin concludes that the saprophytic bacteria of the soil have an antagonistic action towards the typhoid bacillus. This conclusion is not warranted, as the difficulties in the detection of the typhoid bacillus in the presence of a large number of saprophytes are almost insuperable by the methods used. The nutrient broth added to the soil allowed of rapid multiplication of organisms of the "subtilis" group. Flügge (1895) has shown that these organisms grow with enormous rapidity at temperatures above 22° C. when placed under favourable conditions, while below that temperature their growth is comparatively slow. This accounts for the greater ease with which the typhoid bacillus was recovered when the soils were kept at a low temperature (2° C.—12° C.). Martin's further experiments show that the products of putrefaction have a bactericidal action on the typhoid bacillus, but these products only occur in the presence of an abundant supply of nitrogenous material. In an ordinary soil the putrefactive bacteria are in a quiescent condition, a large proportion being present in the form of spores. Exp.

No. 11 shows that the addition of filtered sewage does not supply enough organic material to enable the putrefactive bacteria to exert a harmful influence on the typhoid bacillus. In this case the typhoid bacillus was recovered after 86 days, that is, it survived rather longer than in the control experiment with uncontaminated soil. The progressive increase in the number of saprophytes in Exps. Nos. 13 and 15 is not to be interpreted as evidence of an antagonistic action on their part towards the typhoid bacillus, as the latter died off at exactly the same rate in the sterilised sample. In this connection it may be noted that Pfuhl (1899) found that the typhoid bacillus is capable of growing on potato and spreading into its substance even in the presence of *B. subtilis*.

Whether or not the typhoid bacillus can to any extent multiply in a natural soil has not been definitely determined. There is no evidence of such increase in my experiments, but the inoculations were made with such large numbers of bacilli that a slight growth might not have been noted. The constant decrease in numbers under the conditions of the experiments makes it appear very unlikely that any growth does take place.

The results of others who have investigated the survival of *B. typhosus* in soil may be summarised here. Grancher and Deschamps (1889) inoculated soil in a natural condition with broth cultures of *B. typhosus* and recovered the bacillus after five and a half months.

Almquist (1893) recovered the bacillus from a sterile mixture of sand and dung after "a considerable time," while in pure sand it died out rapidly. No exact data are given; broth cultures were used. Dempster (1894) using small quantities of sterilised soil and emulsions of *B. typhosus* in water recovered the bacillus up to the 18th day. In sterilised peat the bacillus disappeared in 24 hours. He considers that in soils which have not a definite destructive action on the bacillus the time of survival is chiefly dependent on the amount of moisture present.

Robertson (1898) added broth cultures of *B. typhosus* to patches of ground from which the grass had been removed. In one set of observations he recovered the bacillus after four months, in another where frequent additions of nutrient material were made to the soil the bacillus survived for ten months.

Rullman (1901) following Martin's methods showed that the typhoid bacillus can spread in some soils to which it has been added in the form of broth cultures. He recovered the bacillus after 216 days in sterilised, and after 100 days in unsterilised samples.

Lorrain Smith (1903) investigated the survival of *B. typhosus* in soils

from various sources both after sterilisation and in the natural condition. He used emulsions made from growths on potato, care being taken not to add any nutrient material along with the bacilli. He also enumerated the typhoid bacilli present in the samples from time to time using phenolated gelatin plates. The counts showed a rapid diminution in the numbers of typhoid bacilli. The longest period of survival observed in the unsterilised soils was 21 days and the average was 15 days. In the sterilised soils inoculated as mentioned above, the average survival was 16 days and the maximum 25 days. For purposes of comparison samples of sterilised soil to which amounts of nutrient broth varying from 10 % to 0.01 % had been added were inoculated with *B. typhosus*. Here the bacilli died out at the same rate in all the samples and were not recovered after 23 days. The samples in this case were taken from the grounds of Queen's College and were therefore of the same nature as those used by the present writer. The results show a directly bactericidal action of the *sterilised* soil. Unfortunately control experiments with unsterilised soil were not in this case made. Another experiment of Lorrain Smith's is given here in detail for comparison with those of the present paper.

"Experiment 22. Soil from the College grounds dried, sifted, and saturated with sewage; again dried and steam sterilised. It was then inoculated with a culture of typhoid bacilli.

Date	Day	Moisture	No. of bacilli per gramme
29/1/03	1	21.5 %	1,075,000
30	2		105,000
1/2/03	4		27,000
3	6		38,000
5	8		4,300
7	10		8,000
10	13		8,700
12	15		1,200
16	17		840
21	24		550
23	26		435
23/3/03	55		Less than 100 "

The time of survival in this sterilised soil corresponds with that found in Exp. No. 14. In this case previous sterilisation did not render the soil specially unsuitable for the survival of the typhoid bacillus as in Exps. Nos. 3, 4, and 10.

I wish here to express my thanks to Prof. Lorrain Smith for much kind help and advice.

SUMMARY OF CONCLUSIONS.

1. The typhoid bacillus can survive in natural soil in large numbers for about 20 days and is still present in a living condition after 70 to 80 days.

2. There is no evidence that the typhoid bacillus is capable of multiplying and leading a saprophytic existence in ordinary soil.

3. In some samples of soil, but not in all, the typhoid bacillus dies out much more rapidly (in 11 days) if the soil has previously been subjected to sterilisation by steam under pressure. This is apparently due to the production of bactericidal substances during sterilisation.

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OBSERVATIONS ON THE BACTERIOLOGY OF AN
EPIDEMIC OF DIPHTHERIA IN A SCHOOL,
WITH SPECIAL REFERENCE TO THE VIRULENCE OF THE
ORGANISMS WHICH WERE ISOLATED FROM THE CASES.

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IN November 1906 an opportunity occurred to investigate an epidemic of diphtheria occurring at the Duke of York's School, Chelsea. This is a military school of 551 boys, between the ages of 9 and 14 years, and 40 students, or older lads, 17 to 19 years of age. A certain number of both boys and students were affected.

History of the outbreak.

The epidemic of sore throats began about the 26th of August 1906, immediately after the boys had returned from camp at the seaside. Throughout September, October, and the first half of November, cases of sore throat occurred daily with occasional intervals of a day or two. Almost all of the cases presented the clinical features of follicular tonsillitis.

The earliest case which was clinically considered to resemble diphtheria occurred on 20th September, in a boy who had at the time, in addition to sore throat, a free nasal discharge which continued with an interval for at least two months; his nose and throat yielded in November the *Bacillus diphtheriae*.

Usually there was a sudden onset with vomiting and a temperature of 101°—103° F. The illness only lasted four or five days, after which the patient, as a rule, appeared well. In no case was there a rash. No cases of peritonsillar abscess were observed. Altogether 183 boys and students had sore throats.

Albumen was found in the urine of two of the boys who were removed to the Metropolitan Asylums' Board Hospital. But although the urine of many of the patients who remained in the school was examined, in none was albumen found. Paralysis of the fauces was observed in only two cases, and in one of these accommodation was also affected. The general course of the disease was in every case mild, and recovery was uneventful, with the exception given above. All those affected recovered completely.

The epidemic was dealt with in the following manner by Lieut.-Col. Fayrer, the Medical Officer to the Duke of York's School: between 17th and 22nd November 1906, the whole school received a dose of diphtheria antitoxin, 109 boys in whose throats diphtheria bacilli had been found received 1000 units, and the rest 500 units: in addition all those with *B. diphtheriae* were either sent to hospital or isolated.

After the prophylactic injection until the middle of January, only five cases of sore throat occurred, and in none of these was the diphtheria bacillus found. But in one boy in apparent health, a patch of membrane was found on the fauces on 12th December, from which diphtheria bacilli were obtained. The epidemic ended suddenly on the adoption of the plan of isolation of carriers and prophylactic injection.

I first had the opportunity of examining swabs taken from the boys' throats on the 3rd November, and after that I examined all cases of sore throat for *B. diphtheriae*. Swabs taken from the fauces were inoculated the same day on solidified blood serum, and after the tubes had been incubated at 37° C. for about 20 hours, they were examined for *B. diphtheriae*. Its presence or absence was determined by examining films stained by Loeffler's methylene blue.

In order to determine with greater certainty the nature of such strains as were isolated, in addition to observing morphological and cultural characters, experiments with regard to acid production from various carbohydrates, virulence for guinea-pigs, and agglutination were made.

Results of Examinations of Swabs from Throats.

Forty boys were examined whilst actually suffering from sore throat between the 3rd and 18th of November, and of these 15 (= 37 %) yielded *B. diphtheriae*.

On 14th November, Dr Boycott, Dr Marshall and myself, took swabs from the fauces of all the boys in the school who were not in hospital,

and on 19th November 33 students were examined in the same way. On these two occasions 537 boys and students were examined and a positive diagnosis of *B. diphtheriae* was made in 118 cases (= 21 %), all of whom, with one exception, appeared to be in good health. In this case a patch of membrane was found on the fauces at the time when the swab was taken. In addition 182 (= 43%) were found to harbour *B. pseudodiphtheriae* (Hofmann). No attempt was made to count the number of those yielding Hofmann's bacillus in addition to *B. diphtheriae*. The above 182 cases with Hofmann's bacillus were found amongst those not giving *B. diphtheriae*, and the prevalence of Hofmann's bacillus is therefore best indicated by reckoning these cases as a percentage of the 419 cases in which *B. diphtheriae* was not found. Calculated in this way the proportion with Hofmann's bacillus was 43 %.

If we now take the whole school of 591 boys and lads into consideration (including the 40 students of 17—19 years), and the whole time from the beginning of the epidemic in the last days of August 1906 to the middle of January 1907, the following facts emerge :

Total number of boys and students in school	= 591,
Total number who had sore throats	= 183 or 31 %,
Total number found with <i>B. diphtheriae</i>	= 136 or 23 %.

Incidence of B. diphtheriae.

Of 40 boys with sore throat when examined ...	15 or 37 %	had <i>B. diphtheriae</i> .
Of 183 boys who at some time had sore throats	55 or 30 %	„ „
Of 591 boys (i.e. whole school)	136 or 23 %	„ „
Of 408 boys who did not have sore throats ...	81 or 20 %	„ „

Very few examinations were made before the 3rd November.

The boys who suffered from sore throats and those who were proved to harbour *B. diphtheriae* were by no means identical, for although 136 yielded *B. diphtheriae*, only 55 of these had had sore throat.

Characters of the Strains isolated.

Twenty strains of bacilli morphologically resembling *B. diphtheriae* isolated from cases taken haphazard were further examined. Four of these strains were from patients who had clinical symptoms when examined (3 cases of sore throat and one of nasal discharge), the rest were from boys who had no symptoms when examined, but 3 of whom had had sore throats about four weeks previous to the examination.

One of these 20 strains (D 73) morphologically and culturally some-

what resembled *B. coryzae segmentosus*, but a dose of 2 c.c. of a 48-hour broth culture killed a guinea-pig in $4\frac{1}{2}$ days. In addition 4 other diphtheroid strains were isolated; 2 of Hofmann's bacillus (A 12, D 39), and 2 organisms resembling *B. coryzae segmentosus* (G 17, G 36). Certain other strains of diphtheria and diphtheroid bacilli obtained from other sources were subjected to the same tests for the sake of comparison (see footnote to Table I).

Morphology.

As described by other writers, films made from 20-hour cultures on solidified serum and stained by Loeffler's blue showed many variations in the length and structure of the bacillus. All the strains showed beading or segmentation and characteristic grouping.

Three of the strains (B 28, C 49, and Y 11), which were most typical of the long form of the *B. diphtheriae*, were amongst the non-virulent strains. Neisser's stain yielded uncertain results; for instance virulent strains of *B. diphtheriae* (Y 57, St. 28) showed no polar bodies, but diphtheroids (D. 39, Waite) which certainly were not true *B. diphtheriae* had well marked polar bodies.

B. coryzae segmentosus in a film from a 20-hour culture usually appeared as a very short diplococcus-like bacillus, but after 48 hours or more had grown into a long and much segmented form, sometimes with swollen ends. After a few days the beaded forms of the bacillus when stained with Loeffler's blue often closely resembled a chain of streptococci.

None of the 7 strains of non-virulent *B. diphtheriae* isolated in this epidemic resembled those races of diphtheroids described by Gordon (1902) or Graham-Smith (1904) as being related to but not identical with the *B. diphtheriae*.

Acid Production from Sugars.

Liquid media containing 1% of glucose, maltose, galactose, laevulose, lactose, cane sugar and mannite were used in these tests. A series of tests was made with a medium consisting of 25% beef broth and 75% peptone water and litmus. The results are given in Table I. Acid was produced by the strains of *B. diphtheriae* in the presence of all these substances except cane sugar and mannite.

A more complete series was carried out with litmus-peptone-water (2% Witte's peptone in tap water) as the basis of the medium. This

Hiss' medium with 1 % carbohydrates.

Graham-Smith		Knapp									
		<i>B. diphtheriae</i>									
<i>B. diphtheriae</i>	+	+	+	+	+	+	+	+	+	+	+
<i>B. xerosis</i>	+	-	+	+	+	-	+	+	+	+	+
<i>B. coryzae</i>											
<i>segmentosus</i>	+	-	+	-	-	-	+	+	+	+	-
Certain other diphtheroids	+	+	+	-	-	-	+	+	+	+	-
+ S = slightly acid after 48 hours. + = acid after 48 hours. VV = fair virulence. V = low virulence. N = non-virulent.											
The results recorded by Knapp (1904) and Graham-Smith (1906) are included in this Table.											

Sources of *B. diphtheriae* and diphtheroid bacilli examined.

- (1) P. W. The toxigenic strain No. 8 of Park and Williams.
- (2) Bl. 2 } Virulent strains of *B. diphtheriae* from Dr Blumenthal of Moscow; the bacilli are of very long form and often
- (3) Bl. 4 } branched.
- (4) Ble. A bacillus from ear discharge in scarlet fever; thick opaque, whitish growth on agar; stained nearly uniformly by methylene blue.
- (5) By. From discharge from the middle ear. It resembled 'Ble.' macroscopically; when stained by methylene blue it showed regular transverse bands.
- (6) W From the vaginal discharge of a child. It grew feebly in very small colonies on serum and agar. Uniformly barred when stained by methylene blue.
- (7) 18 (2) } Diphtheroids of the type of *B. coryzae segmentosus* from catarrhal noses.
- (8) 21 (1) }

TABLE II. *Peptone water (2%) with 1% carbohydrates.*

Strains of <i>B. diphtheriae</i>	Viru- lence	Glucose	Lae- Maltose	Cane sugar	Lactose	Mannite	Galac- tose	Strains of <i>B. diphtheriae</i>	Viru- lence	Glucose	Lae- Maltose	Cane sugar	Lactose	Mannite	Galac- tose
St. 28	VV	+	+	-	-	-	+	E 6	N	+	+	-	-	-	+
A 67	VV	+	+	-	-	-	+	St. 33	N	+	+	-	-	-	+
St. 29	VV	+	+	-	-	-	+	St. 22	N	+	+	-	-	-	+
E 25	VV	+	+	-	-	-	+	B 28	N	+	+	-	-	-	+
D 3	VV	+	+	-	-	-	+	Y 11T	N	+	+	-	-	-	+
G 50	VV	+	+	-	-	-	+	C 49	N	+	+	-	-	-	+
Y 3	VV	+	+	-	-	-	+	St. 17	N	+	+	-	-	-	+
A 37	V	+	+	-	-	-	+	Strains of <i>B. pseudo-diphtheriae</i> (Hofmann):							
Y 35	V	+	+	-	-	-	+	A 12	N	-	-	-	-	-	-
Y 57	V	+	+	-	-	-	+	D 39	N	-	-	-	-	-	-
G 61	V	+	+	-	-	-	+	Strains of <i>B. coryzae segmentosus</i> :							
E 71	V	+	+	-	-	-	+	G 17	N	+	-	-	-	-	+S
D 73	V	+	+	-	-	-	+	G 36	N	+	S	-	-	-	+S
Strains from other sources:								Strains of other diphtheroids from outside sources:							
P. W.	VV	+	+	-	-	-	+	Ble.	N	+	-	+	-	-	+
Bl. 2	V	+	+	-	-	-	+	By.	N	+	-	+	-	-	+
Bl. 4	VV	+	+	-	-	-	+	W	N	+	-	+	-	-	+
								18 (2)	N	+	-	-	-	-	+
								21 (1)	N	+	-	-	-	-	-

VV = killed guinea-pig in dose of 0.1 c.c.

V = " " 2.0 c.c.

N = did not kill guinea-pig in dose of 2.0 c.c.

+ = acid.

+ S = slightly acid.

- = not acid.

series (Table II) gave uniform results which were different from the previous series (Table I) in that acid was never produced from lactose. In the tubes containing cane sugar and mannite, acid was never produced in either series. The growth was good in all the tubes.

It was thought that the muscle sugar in the beef broth might account for the acidity in the lactose tubes of the first series, although no acid had appeared in the presence of cane sugar or mannite. Accordingly 12 strains were tested again in three different media :

- (1) Weak litmus broth as above, without lactose.
- (2) Weak litmus broth as above, with 1 % lactose.
- (3) Peptone water with 1 % lactose.

The result is shown in Table III.

TABLE III.

<i>Bacillus diphtheriae</i>	Virulence	Weak broth, no lactose		Weak broth, 1 % lactose		Peptone water 1 % lactose
		2 days	7 days	2 days	7 days	
St. 28	VV	+ S	S	+	++	-
A 67	VV	+ S	+ S	+	++	-
E 25	VV	+	+	+	++	-
D 3	VV	-	-	-	-	-
Y 3	VV	+ S	+	+	++	-
Y 35	V	+ S	+ S	+	++	-
Y 57	V	+ S	+ S	+	++	-
C 49	N	+ S	-	+	+ S	-
St. 17	N	+ S	-	+	++	-
P. W.	VV	-	-	S	VS	-

Diphtheroids from otorrhoea :

Ble.	V	-	-	+	++	-
By.	N	-	-	S	-	-

VV = virulent.

V = low virulence.

+ S = slightly acid.

+ = acid.

++ = very acid.

- = neutral or alkaline.

The supposition was not found to be justified, for as before, the peptone water with 1 % lactose remained uniformly neutral; the weak broth without lactose was usually slightly acid after one or two days but later became less acid or alkaline; the weak broth with 1 % lactose soon became acid and later a strong acid reaction developed in it.

Virulence.

The virulence of each strain was tested by injecting subcutaneously into three guinea-pigs (about 250 grammes) doses of 0·1 c.c., 2·0 c.c., and 2·5 c.c. respectively of a 48-hour culture in alkaline broth, 0·1 c.c. of antitoxin having been previously added to the 2·5 c.c. dose. The animal which received antitoxin along with the culture remained well in every case. The results are given in Tables IV and V below.

TABLE IV. *Virulence for the guinea-pig of the isolated strains.*

Strains	Dose of a 48 hrs. culture in alkaline broth		Organism	Occurrence of sore throat	Strains	Dose of a 48 hrs. culture in alkaline broth		Organism	Occurrence of sore throat
	2·0 c.c.	0·1 c.c.				2·0 c.c.	0·1 c.c.		
St. 28	†17 hrs.	†36 hrs.	Bac. d.		E 6	—	—	Bac. d.	
A 67	†36 „	†36 „	„	P	St. 33	—	—	„	
St. 29	†36 „	†36 „	„		St. 22	—	—	„	
E 25	†12 „	†84 „	„		B 28	—	—	„	
D 3	†60 „	†6 days	„		Y 11T	—	—	„	P
G 50	†84 „	†8 „	„		C 49	—	—	„	
Y 3	†20 „	†10 „	„	E	St. 17	—	—	„	
A 37	†19 „	—	„		A 12	—	—	H	
Y 35	†36 „	—	„	E	D 39	—	—	H	
Y 57	†36 „	—	„	E	G 17	—	—	B. C. S.	
G 61	†36 „	—	„	P	G 36	—	—	B. C. S.	
E 71	†36 „	—	„	P					
D 73	†4½ days	—	„						

Bac. d. = *Bacillus diphtheriae*.

P = previously.

H = „ *pseudo-diphtheriae* (Hofmann).

† = death.

B. C. S. = „ *coryzae segmentosus*.

— = lived.

E = at time of examination.

E and P in the 5th column refer to the occurrence of sore throat in the patient from whom the strain was isolated.

TABLE V. *Proportion of Virulent and Non-virulent Strains.*

4 Strains	Killed in dose of		Glucose reaction	Morphology	Percentage
	0·1 c.c. within	2·0 c.c. within			
3 „	4 days	48 hours	Acid	Bac. d. }	35
6 „	10 „	4 days	„	„ }	30
7 „	—	4½ „	„	„ }	35
20	—	—	„	„ }	

Bac. d. = *Bacillus diphtheriae*.

Of the 4 strains regarded on other grounds as *B. pseudo-diphtheriae* (Hofmann) and *B. coryzae segmentosus*, none caused death.

Of the 20 strains from the school resembling *B. diphtheriae* morphologically, only four killed within 4 days, when a dose of 0·1 c.c. was

injected; one in 8 days and one other in 10 days; and six strains killed in a dose of 2.0 c.c. leaving seven strains (35%) quite devoid of killing power.

If the 20 strains isolated can be taken as a fair sample of the rest, then 7% of the boys in the school harboured non-virulent *B. diphtheriae*.

In order to make sure that the defective virulence was not due merely to the sample of broth used or to a weak, but not entirely absent, power of forming toxin, 4 non-virulent strains were grown for 10 days in a special alkaline broth which contained 2% peptone and which had been found very efficient for making toxin. The same doses as before were injected into guinea-pigs, but in no case did death result.

Agglutination.

To obtain further evidence which should decide the question as to the relationship of the virulent and non-virulent strains of diphtheria bacilli, I applied an agglutination test. For this test I employed the serum of a horse which had been immunised some time before, with the view to testing the value of anti-microbial serum.

In the process of immunisation one race of *B. diphtheriae* was employed, viz. the toxigenic bacillus No. 8 of Park and Williams. At the outset of the immunisation intravenous injections of killed cultures of the bacillus were employed, later the living bacilli were used.

The agglutination results (Table VI) were not uniform for the virulent strains, but the majority of these, 10 in number (including the strains from outside sources), gave positive results. One gave a slight, one a doubtful result, and one was negative; three were not tested.

Of the non-virulent diphtheroids, the two strains of *B. pseudo-diphtheriae* (Hofmann) and the two strains 'Ble.' and 'By.' from ear discharges gave negative results.

Two non-virulent diphtheroids (W and 18 (2)) gave doubtful results; one (21 (1)) gave a negative result.

Of six non-virulent diphtheria bacilli from this epidemic, four gave slight positive, and two negative results.

The agglutination tests indicate differences in the races of virulent diphtheria bacilli, and also a divergence of the non-virulent strains from the majority of virulent strains.

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TABLE VI. Agglutination Macroscopic.

Strain	Viru- lence	Immune serum				Normal horse serum				Salt soln.	Immune serum				Normal horse serum				Salt soln.
		1/20	1/50	1/100	1/200	1/20	1/50	1/100	1/200		1/20	1/50	1/100	1/200	1/20	1/50	1/100	1/200	
St. 28	VV	++	++	++	S	++	++	++	S	S	++	++	++	+	++	++	++	+	S
A 67	VV	++	++	++	S	++	++	++	S	S	++	++	++	+	++	++	++	+	S
St. 29	VV	++	++	++	S	++	++	++	S	-	++	++	++	+	++	++	++	+	S
E 25	VV	++	++	++	S	++	++	++	S	-	++	++	++	+	++	++	++	+	S
D 3	VV	++	++	++	S	++	++	++	S	+	++	++	++	+	++	++	++	+	S
G 50	VV	++	++	++	S	++	++	++	S	+	++	++	++	+	++	++	++	+	S
Y 3	VV	++	++	++	S	++	++	++	S	+	++	++	++	+	++	++	++	+	S
A 37	V	++	++	++	S	++	++	++	S	+	++	++	++	+	++	++	++	+	S
Y 35	V	++	++	++	S	++	++	++	S	+	++	++	++	+	++	++	++	+	S
G 61	V	++	++	++	S	++	++	++	S	+	++	++	++	+	++	++	++	+	S
E 71	V	++	++	++	S	++	++	++	S	+	++	++	++	+	++	++	++	+	S
D 73	V	++	++	++	S	++	++	++	S	+	++	++	++	+	++	++	++	+	S
From other sources:																			
P. W.	VV	++	++	++	S	++	++	++	S	-	++	++	++	+	++	++	++	+	S
Bl. 2	V	++	++	++	S	++	++	++	S	-	++	++	++	+	++	++	++	+	S
Bl. 4	VV	++	++	++	S	++	++	++	S	-	++	++	++	+	++	++	++	+	S

Strains from other sources not *B. diphtheriae*.

+++, ++, +, +S, S and VS = different degrees of sedimentation in the tubes.

++ = complete sedimentation.

- = no sedimentation.

The Occurrence of Bacillus fusiformis and Spirochaetes.

Another interesting feature of the epidemic was the frequent occurrence in the cases of sore throat of the *B. fusiformis* and of spirochaetes. These organisms were seen in smears made direct from the throat. They were found chiefly in cases in which *B. diphtheriae* was also present.

Of 45 cases of sore throat examined 20 showed *B. fusiformis* and 14 also yielded spirochaetes, both of which were often present in very large numbers in smears made at once from swabs taken from the fauces. Of the 16 cases of clinical diphtheria in which *B. diphtheriae* was found, 11 had also *B. fusiformis* and 10 spirochaetes as well as *B. fusiformis*. If the swabs had been taken a few hours or allowed to dry before the smears were made, *B. fusiformis* and spirochaetes were seldom found, and if found, were present in small numbers.

The frequent occurrence of *B. fusiformis* and spirochaetes on the fauces of patients suffering from diphtheria has been described by Priestley (1906) and Leiner (1906). Leiner considers the coexistence of *B. diphtheriae*, fusiform bacilli and spirochaetes to be quite common, and says that they are found associated mainly in two distinct groups of clinical diphtheria: the one, of very severe—the so-called septic type—the other of milder character with slight general symptoms.

Remarks.

The large number (21 %) of boys in the school who were found to harbour *B. diphtheriae* in the fauces, and the large proportion of the strains of bacillus which proved to be either non-virulent or of low degree of pathogenicity for guinea-pigs, is remarkable. Non-virulent strains were estimated to be present in 7 % of all the boys. The presence of bacilli in the throats of 4 to 10 % of apparently healthy children, in connection with outbreaks of diphtheria, has been recorded by Graham-Smith, Thomas, Ustvedt, Pennington, and others.

Graham-Smith (1904) during an epidemic of diphtheria at Cambridge found diphtheria bacilli in the fauces of 3.9 % of the children who were contacts (in 2.6 % virulent and in 1.3 % non-virulent strains) and taking the notified cases and contacts together, in 8.2 % (in 6.4 % virulent, in 1.8 % non-virulent) of the children examined.

Thomas (1905) found from the examination of those children in London schools, who without obvious symptoms of diphtheria were suspected of spreading the disease, that 6.7—7.3 % harboured diphtheria bacilli.

Ustvedt (1906) found that in infected schools in towns, bacilli morphologically indistinguishable from diphtheria bacilli, were obtained from 4·5 % of the children. In an uninfected country district, none could be found amongst the school children; they were however present in the fauces of 14·21 % of those in close contact with patients.

Pennington (1907) examined the fauces of a large number of apparently healthy school children in Philadelphia. He obtained bacilli resembling *B. diphtheriae* from about 10 % of the children and of the strains isolated 35—50 % showed no virulence for guinea-pigs, and 14 % killed guinea-pigs fairly promptly, whereas 30 % he considers showed some pathogenicity, but were of attenuated virulence for guinea-pigs.

Where the observations have been made from cases distributed over a town or district, no relation has been established between the severity of an attack of diphtheria and the virulence of the bacilli for guinea-pigs (Smith and Walker (1896), and Richmond and Salter (1898)).

Cobbett (1901) and Graham-Smith in an epidemic in which cases of very different degrees of severity occurred, found that the strains isolated were of a singularly uniform degree of virulence. They obtained no evidence in favour of the possibility of attenuation of the bacillus.

There is more likelihood, however, that an epidemic within a small area, as in the present case, may have originated from a single strain of bacillus. The low degree of virulence of most of the strains which were pathogenic for guinea-pigs and the high percentage of non-virulent strains associated with a large number of cases of a very mild character can be best interpreted by the assumption that the outbreak in this school was due to an attenuated strain of the *B. diphtheriae*.

The agglutination test which I have used has yielded variable results with virulent and non-virulent strains, but on the whole has tended to accentuate the difference between the virulent and the non-virulent. Other observers have used agglutinating sera for examining strains of *B. diphtheriae* (Lubowski (1900) and Gordon (1901–2)).

Summary and Conclusions.

(1) A localised epidemic of diphtheria of a clinically mild type (both as regards local and general symptoms) was associated with the prevalence in the fauces of patients and contacts of strains of *B. diphtheriae* of low pathogenicity for animals; 35 % of these strains possessed moderate virulence for guinea-pigs, 30 % low virulence and 35 % were non-virulent.

(2) Prolonged nasal discharge after moderately severe diphtheria was in one case associated with a strain of *B. diphtheriae* which was non-virulent for guinea-pigs.

(3) Acid production in various carbohydrate media proved a valuable means of differentiating diphtheria-like bacilli, but the exact composition of the medium is of importance. Peptone water appears to be especially suitable for the basis as being least liable to variation.

(4) The agglutination test gave a fairly uniform result with most of the strains of virulent *B. diphtheriae*, but three virulent strains did not give a decided positive reaction to this test. Of the non-virulent strains half gave a slight, the remainder a negative result.

Bacillus pseudo-diphtheriae (Hofmann) and certain acid producing diphtheroids gave no reaction.

(5) *B. fusiformis* and spirochaetes occurred in large numbers on the fauces in this epidemic of diphtheria, in association with the *B. diphtheriae*.

(6) After all the boys had received prophylactic injections of antitoxin and after the carriers had been isolated the epidemic promptly ceased.

I have to thank Lieut.-Col. Sir Joseph Fayrer, Medical Officer of the Duke of York's School, who was at the time Acting Commandant, for his permission to publish this account.

I am very much indebted to Dr Boycott and Dr Marshall for the very large amount of help which they gave me, and to Dr Dean for his assistance throughout the investigation.

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ON THE RELATIVE EFFICACY OF THE DOULTON, BERKEFELD AND BROWNLOW FILTERS.

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(Two Figures in the text.)

IN a previous communication (1906) it was shown by Bulloch and Craw that the Doulton "white filter" prevented the direct transmission of micro-organisms, and that the filtrates from highly contaminated waters were germ free until sufficient time had been given for the bacteria to grow through the filter mass. It was demonstrated, in fact, that the Doulton filter was at least as efficient in the retention of micro-organisms as the best material on the market, viz. the Pasteur-Chamberland filter, and excelled the latter in its rate of filtration. It was pointed out that, with the possible exception of *Kieselguhr* filters, only porcelain bougies could be relied upon to prevent direct transmission of germs. During the past year we have tested the relative efficiency of porcelain and *Kieselguhr* filters, in particular the Doulton porcelain filter and the Berkefeld *Kieselguhr* filter. A few samples of the Slack and Brownlow filter (sand and porcelain mixture?) were also tested.

The Berkefeld filter has been repeatedly tested by bacteriologists in the last fifteen years, and for a time, at any rate, it was considered to be an efficient filter. Fourteen years ago, however, M. Kirchner (1893) had shown that it was not a trustworthy filter, a view more recently

held by E. Pfuhl (1903). In a series of experiments carried out by him 50% of the large Berkefeld candles were unable to keep back bacilli approximating the size of *B. typhosus* and *B. dysenteriae*. With the small Lilliput-*Kieselguhr*-filter the results were even more disappointing, as of four candles tested only one yielded a sterile filtrate of 100 c.c. Of the remaining three one yielded only 50 c.c. of sterile filtrate, the other two were even worse. The matter is of considerable importance in connection with filtration experiments as an indication of the ultra-microscopic size of the microbes of various diseases, for it has been frequently supposed that if the filtrate, from an infectious material which has traversed a Berkefeld filter, is infective, the infecting agent must be ultra-microscopic. Novy and Knapp (1906) have, however, shown that so large a microbe as *Spirochaeta obermeieri* (7 to 19 μ or more in length) easily traversed the small Berkefeld filters (35 mm. long \times 15.5 mm. broad) under a pressure of 50 pounds within 10 minutes of the commencement of the experiment. Novy and MacNeal (1904) also found that even *Trypanosoma brucei* can pass through a Berkefeld filter.

On the other hand it will be remembered that in their exhaustive inquiry Woodhead and Cartwright Wood (1894, 1898) stated that Berkefeld filters "afford complete protection against the communication of water-borne disease."

With reference to the Slack and Brownlow filters the chief experiments are those of Woodhead and Cartwright Wood (1898), who also considered that this filter "protects against the transmission of water-borne disease." The Board of Trade (Marine Department) also states that "Slack and Brownlow's filters have undergone stringent tests, and may be passed as part of the medical stores required by the Board's medical scale for emigrant and merchant ships."

The following experiments were carried out to test the relative efficacy of the Doulton, the Berkefeld and the Brownlow germ filters.

In order to prevent any unfair comparison all the filters used were obtained in the public market.

A full account of the earlier investigations of water filters, with references, is given by Loeffler and Oesten in Weyl's *Handbuch der Hygiene* (1896), Bd. I. Abt. 2.

Method.:—Figures 1 and 2 give a diagrammatic representation of the arrangement used in the comparison of the various filters. To the water main, *M*, a vertical branch pipe, *B*, was fitted carrying a horizontal pipe, *Hr*, to which a Bourdon pressure gauge, *G*, was affixed.

A T-piece was attached to the horizontal portion furnished with screw taps, *T*, *T'*, on its remaining limbs.

This arrangement permitted of the direct comparison of two different kinds of filters under identical conditions as regards degree of contamination and variation in pressure of the water supply, etc.

In the figures a Doulton filter, *D*, and a Berkefeld, *Bk*, in their metallic cases, *C*, *C'*, are shown attached to these taps by the rubber lined hoods or sockets, *H*, *H'*.

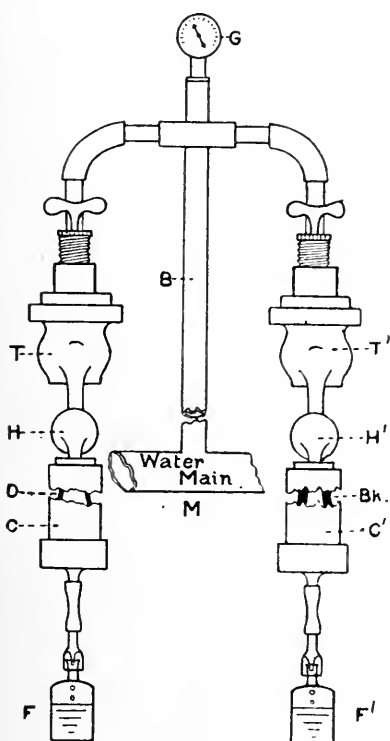


Fig. 1.

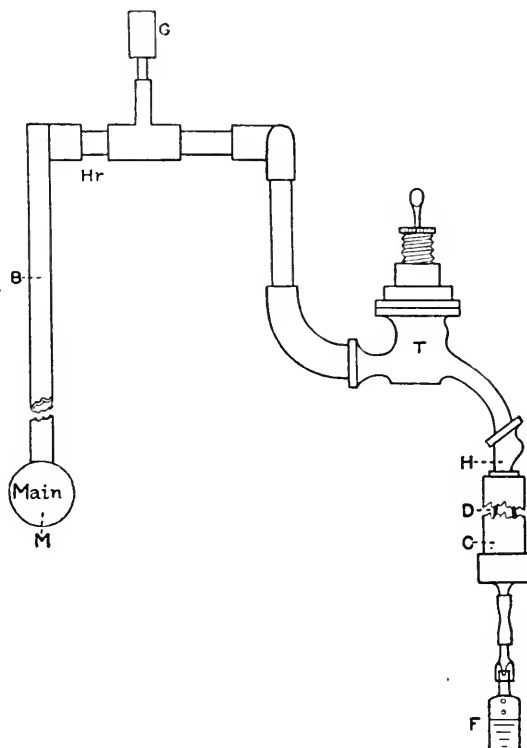


Fig. 2.

Hooded glass nozzles were connected by rubber to the exits of the filters in order that the samples of filtrate taken in the flasks, *F*, *F'*, should escape contamination by air organisms. The filters, together with the glass nozzles, were sterilised by heating to 120° C. for one hour in the metal cases supplied by the makers. After attaching to the sockets or hoods, *H*, *H'*, the water was screwed full on and allowed to run continuously. Samples of the filtrates from the two filters to be compared were collected simultaneously every few hours in bottles containing a solution of 10% peptone and 5% salt, so as to give in the test sample about 1% peptone. These samples were incubated at 37° C. for over seven days, and the day noted on which the first trace of growth appeared. In many cases the clear and the cloudy

fluids were also examined microscopically and the clear fluids were uniformly found to be germ free, whereas the cloudy samples were rich in micro-organisms. The tap water taken directly from the main was found to contain an average of 50 to 100 germs per c.c., as indicated by cultivation on agar plates, the examinations being made as soon as the samples had been taken.

Examination for Transmission of Bacteria from Tap Water.

Tables I, II, and III clearly indicate the relative efficiencies of the Doulton, the Berkefeld and the Slack and Brownlow filters. Tests of the Doulton filter, ten in number, ten of the Berkefeld and five of the Slack and Brownlow were made under identical conditions.

TABLE I. *Tests of ten Doulton Filters for transmission of micro-organisms under variable pressure.*

		Maximum pressure 32.5 lbs. per sq. inch.						
		Dimensions of filters—diameter, base 3.2 cm., apex 2.3 cm., length 18 cm.						
No.	Day of filtration—	1	2	3	4	5	6	7
1	Growth	0	0	0	+	+	—	—
	Day of incubation	7	7	7	7*	7	—	—
2	Growth	0	0	0	+	+	+	+
	Day of incubation	7	7	7	7*	7	7	7
3	Growth	0	+	+	+	+	—	—
	Day of incubation	7	7*	7	7	7	—	—
4	Growth	0	0	0	—	+	+	—
	Day of incubation	7	7	7	—	2	1	—
5	Growth	0	0	0	—	0	+	—
	Day of incubation	7	7	7	—	7	2	—
6	Growth	0	0	0	+	—	—	—
	Day of incubation	7	7	7	2	—	—	—
7	Growth	0	0	+	+	+	—	—
	Day of incubation	7	7	2	1	1	—	—
8	Growth	0	+	—	—	—	—	—
	Day of incubation	7	2	—	—	—	—	—
9	Growth	0	0	0	0	+	—	—
	Day of incubation	7	7	7	7	3	—	—
10	Growth	0	0	0	+	—	—	—
	Day of incubation	82	81	80	1	—	—	—

* Growth may have appeared before the 7th day of incubation.

0 represents no growth. + represents growth. — observation not made.

Doulton. From Table I it will be seen that only two out of ten Doulton filters transmitted germs on the second day of continuous

filtration, and the incubation periods, viz. two and seven days, indicate that the number of germs must have been very small. In no case did the filtrates from the Doulton filters show the slightest sign of contamination during the first 24 hours, which shows that no direct transmission of organisms took place.

Of the ten filters, one gave slightly contaminated filtrates on the third day.

Four filters transmitted germs on the fourth day, one gave a filtrate on the fifth day which only showed contamination after two days' incubation, and another only after three days at 37° C. Lastly, one filter gave sterile filtrates for five days, and on the sixth day the samples only showed growth after two days' incubation.

TABLE II. *Tests of ten Berkefeld Filters for transmission of micro-organisms under variable pressure.*

Maximum pressure 32·5 lbs. per sq. inch.
Dimensions of filters—diameter 5 cm., length 12 cm.

No.		Day of filtration—1	2	3	4
1	Growth	+	+	+	+
	Day of incubation	1	1	1	1
2	Growth	+	+	+	—
	Day of incubation	1	1	1	—
3	Growth	+	+	+	+
	Day of incubation	1	1	1	1
4	Growth	+	+	+	+
	Day of incubation	1	1	1	1
5	Growth	+	+	+	—
	Day of incubation	1	1	1	—
6	Growth	+	+	—	—
	Day of incubation	1	1	—	—
7	Growth	+	+	—	—
	Day of incubation	1	1	—	—
8	Growth	+	+	—	—
	Day of incubation	1	1	—	—
9	Growth	0	+	—	—
	Day of incubation	7	1	—	—
10	Growth	+	+	—	—
	Day of incubation	1	1	—	—

*Berkefeld*¹. Of ten Berkefeld filters only one gave a sterile filtrate on the first day. The remaining nine gave contaminated filtrates within 15 minutes, that is to say, as soon as the filters were started.

¹ Cf. Table II.

Further, the number of germs passing these nine filters immediately must have been relatively great as growth took place after incubating for one day. These results agree with tests made by Pfuhl (1903).

*Slack and Brownlow*¹. Five of these filters gave uniformly highly contaminated filtrates during the first 15 minutes' filtration, abundant growth taking place after 12 to 24 hours' incubation. Another filter did not allow water to pass owing to the outlet of the candle being blocked by cementing metal.

Note. In order to make sure that the absence of growth on the seventh day of incubation in the case of the Doulton filtrates implied absolute sterility, many of these samples were incubated for 14 days, and in the case of No. 10 filter for over 80 days—at the end of these periods no trace of growth could be detected.

TABLE III. *Tests of five Slack and Brownlow Filters for transmission of micro-organisms under variable pressure.*

Maximum pressure 32·5 lbs. per sq. inch.
Dimensions of filters—diameter 3 cm., length 18·5 cm.

No.		Day of filtration—1	2	3
1	Growth	+	+	+
	Day of incubation	1	1	1
2	Growth	+	—	—
	Day of incubation	1	—	—
3	Growth	+	—	—
	Day of incubation	1	—	—
4	Growth	+	—	—
	Day of incubation	1	—	—
5	Growth	+	—	—
	Day of incubation	1	—	—

Conclusions.

Of the filters tested the Doulton filters alone uniformly prevented the direct transmission of micro-organisms; the Berkefeld filters all permitted of direct transmission, with one exception, and all the Slack and Brownlow filters gave contaminated filtrates immediately. In the cases of the Berkefeld filters and the Slack and Brownlow filters the germs passed through within 15 minutes from the commencement of filtration, whereas seven out of ten Doulton filters withheld the organisms and gave sterile filtrates for four days, and in three cases for longer periods. As these tests were made with a water pressure

¹ Cf. Table III.

varying from zero to 32·5 lbs. per square inch often in a few seconds. the above examination appears to us to be not only severe but also conclusive.

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ON THE GRAIN OF FILTERS AND THE GROWTH OF BACTERIA THROUGH THEM.

WITH REFERENCE TO THE DOULTON, PASTEUR, BERKEFELD
AND SLACK AND BROWNLOW FILTERS.

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Plates I and II.

PLAGGE in 1886 observed that but few water filters prevented the direct transmission of micro-organisms and that all gave contaminated filtrates after being a few days in operation. This result has been widely confirmed, especially by Woodhead and Wood (1894, 1898), and by Bulloch and Craw (1906). Plagge considered that in the case of those filters which did not permit of the immediate or direct transmission of germs the subsequent failure of the filters was due to the gradual growth of the organisms through the filter mass, a view which has been adopted by v. Esmarch (1902) and is generally accepted.

It did not seem to me, however, to be proven that indirect or delayed transmission was entirely due to growth through the filter, and the following investigation was made to determine whether a mechanical acceleration is caused by the water current sweeping the micro-organisms through the filter mass.

As numerous data had already been collected by Bulloch and Craw (1906) and Bulloch, Craw and Atkin (1908) on the filtration of contaminated water through porcelain and Kieselguhr filters under pressure, it was only necessary to determine the rate of transmission of a highly motile germ when the pressure on both sides of the filter was the same.

Work having a bearing in this connection has already been carried out by Cambier (1901) who used the Chamberland "*F*" filter for the isolation of *B. typhosus*. He filled the bougie with bouillon and placed it in a tube of bouillon. On inoculating the interior of the filter and incubating at room temperature the bacillus either did not come through or did so only after many days. If, however, he incubated at 37—38° C., the bacillus came through the filter mass "in a short time," e.g. 1½ days. Kirsch (1903) found that the shortest period for transmission through the Chamberland filter was 13 hours, but often *B. typhosus* required days to penetrate, and Bienstock (1903) had made similar observations.

In the following experiments the Doulton, the Berkefeld and the Slack and Brownlow filters have been compared with regard to their relative transmission of *B. pyocyaneus* and *B. prodigiosus*.

Method. The filter was placed in a large test tube containing sufficient neutral broth to cover two-thirds of the filter mass. The nozzle which protruded from the test tube and the top of the latter were bound with cotton wool and the nozzle exit similarly plugged. The whole was then heated in the autoclave to 120° C. for one hour and subsequently incubated for four days at 37° C. to prove sterility of the bougie and medium. 1 c.c. of a strong broth culture of *B. pyocyaneus* was then introduced into the interior of the bougie and daily observations of the external medium were made, during incubation at 37° C., for the first visible trace of fluorescence and cloudiness. A more extended series of experiments was made in exactly the same manner using *B. prodigiosus* as the infecting organism. Every few days a loopful of the contents of the bougies was withdrawn and tested by incubation at 37° C. with broth to ascertain whether the interior of the candle contained living bacteria.

Examination for growth of B. pyocyaneus.

Doulton filters, Nos. (1), (2), (3) and (4) were tested and did not show any trace of contamination in the external medium until 14, 18, 17 and 7 days, in the respective cases, had elapsed after the time of inoculation.

Berkefeld filters, Nos. (1) and (2) were highly contaminated after three days and two days respectively. In the case of No. (2) growth probably took place earlier, no observations having been made until the end of three days.

Examination for growth of B. prodigiosus.

A set of eight *Doulton filters* was compared with four *Berkefeld filters* and four *Slack and Brownlow filters* under the same conditions.

Of the *Doultons* one only gave trace of growth after one day, two filters showed contamination after three days, one after four days, one after seven days, one after eight days, one after ten days, and one did not give the slightest sign of growth until 29 days had elapsed. The filter noted as being contaminated after ten days had only a very faint opalescence which remained constant in intensity for 36 days notwithstanding the fact that the interior of the bougie contained a rich culture of living bacteria.

All four *Berkefelds* were highly contaminated after one day and all four *Slack and Brownlow filters* showed a very rich growth after one day. To ascertain whether the Doulton filter maintained its great superiority over the Berkefeld and the Slack and Brownlow filters with uniformity, a further batch of six *Doulton filters* was tested and at the moment of writing this paper only one is slightly contaminated, the period of incubation for penetration having been 14 days—the remaining five filters show no trace of growth. Thus out of 14 Doulton filters only one gave contamination approaching that obtained by the Berkefeld and by the Slack and Brownlow filters.

Conclusion. As no porcelain or other filter which I have tested under pressure has given sterile filtrates for more than a week, and as many of the Doulton filters showed sterility of the test medium, when growth through the filter mass took place in the absence of pressure, for much longer periods, I conclude that the so-called “indirect contamination” is not due to growth through the filter alone, but is highly dependent upon the current of fluid passing through. The Doulton filter further proved itself superior to the Berkefeld and the Slack and Brownlow filters in preventing the growth of micro-organisms through the filter mass.

On the grain of the filters examined.

The above investigation seemed to indicate that there is no essential difference between direct contamination and the so-called “indirect contamination” so far as the filters tested are concerned, especially when the filtration takes place under pressure. The differences in time of contamination of the filtrate may depend upon two factors at least:

- (1) The chemical natures of the filter mass and the material subjected to filtration,
- (2) The physical configuration or micro-structure of the filter mass and the physical magnitude of the contaminating matter.

It seems to me to be highly probable that in the filtration of fluids containing colloidal substances, suspended matter, or micro-organisms, the chemical nature of the filter mass will be eliminated very rapidly as a factor in the efficiency of the filter, owing to the formation of a coating of foreign material, derived from the fluid, over the chemically active surface. On the other hand the size of the constituent grains of the filtering material must condition the porosity and consequently the permeability to micro-organisms in a high degree so far as direct transmission is concerned, and probably also, in the above experiments, conditions the rate of growth through the filter, as larger pores will permit of a quicker diffusion and convection of nutrient medium. That the physical nature is the chief factor governing the efficiency of the filter seems to be admirably confirmed on comparing the above results for growth through filters with the micro-photographs (Plate II, Figs. 1 to 4) of thin translucent sections of the filter masses.

The size of pore—the lighter portions in the photographs—is very small in the Doulton filter, relatively greater in the Pasteur-Chamberland, very much greater in the Berkefeld, and in the case of the Slack and Brownlow filter the pores are of striking magnitude. Each of the photographs represents magnification of 100.

The order in which the filters arrange themselves as regards size of pore, or grain, is exactly the same as the order of arrangement for growth of bacteria through them, *e.g.* the Doulton filter shows the smallest grain and gives the least growth. I conclude therefore that the grain of a filter is a very important if not the most important factor in governing the growth of bacteria through the filter mass.

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EXPLANATION OF PLATES.

PLATE I.

PHOTOGRAPHS OF GROWTH.

- Fig. 1 is a comparison of two Doulton tubes, one Berkefeld and one Slack and Brownlow after inoculation with *B. prodigiosus* and incubation for two days at 37° C. The Slack and Brownlow filter (S) was highly contaminated, the Berkefeld (B) markedly, and the two Doulton filters (D, D) not at all.
- Fig. 2, two Doulton filters (D) inoculated with *B. prodigiosus* and incubated for 11 days, one Doulton (D) incubated for 18 days, in the centre of the figure, and two Berkefeld filters (B), at the extreme sides of the figure, incubated for three days only at 37° C. indicates contamination (opalescence) in the cases of the Berkefeld and sterility (transparency) in all the Doulton filters.
- Fig. 3 represents two Doulton tubes A and B of Fig. 2 after incubation for 32 and 25 days respectively with *B. prodigiosus*. A faint opalesence was present in the 32 day tube which was certainly not due to micro-organisms, for it remained constant from the 10th till the 40th day.
- Fig. 4 shows two Doulton and one Berkefeld filters inoculated with *B. pyocyaneus* and incubated for 11 days at 37° C. The lower end of the Berkefeld bougie is nearly on a level with the lower ends of the Doulton filters, but owing to the luxuriant growth of micro-organisms through the former (B) its outline is obscured. The Doultons (D, D) on either side are quite transparent and showed no trace of growth.

PLATE II.

MICRO-PHOTOGRAPHS OF GRAIN.

Thin Translucent section magnified to 100 diameters.

- Fig. 1, Doulton White filter tube.
Fig. 2, Pasteur-Chamberland filter tube.
Fig. 3, Berkefeld filter tube.
Fig. 4, Slack and Brownlow filter tube.

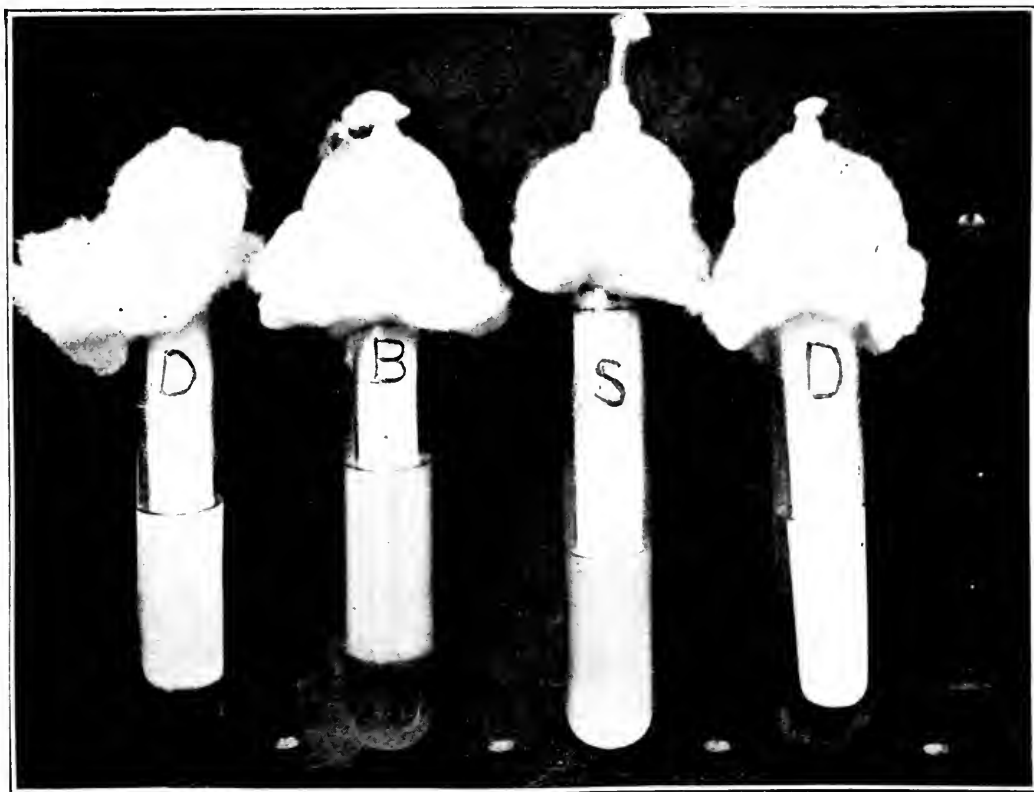


Fig. 1.

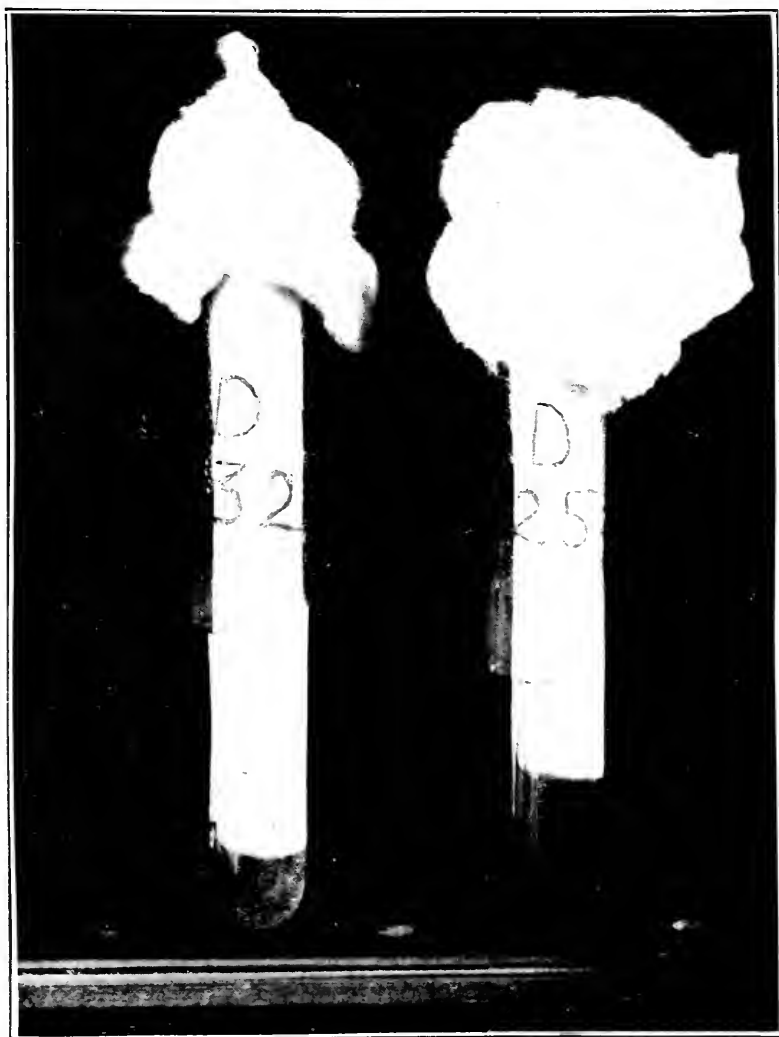
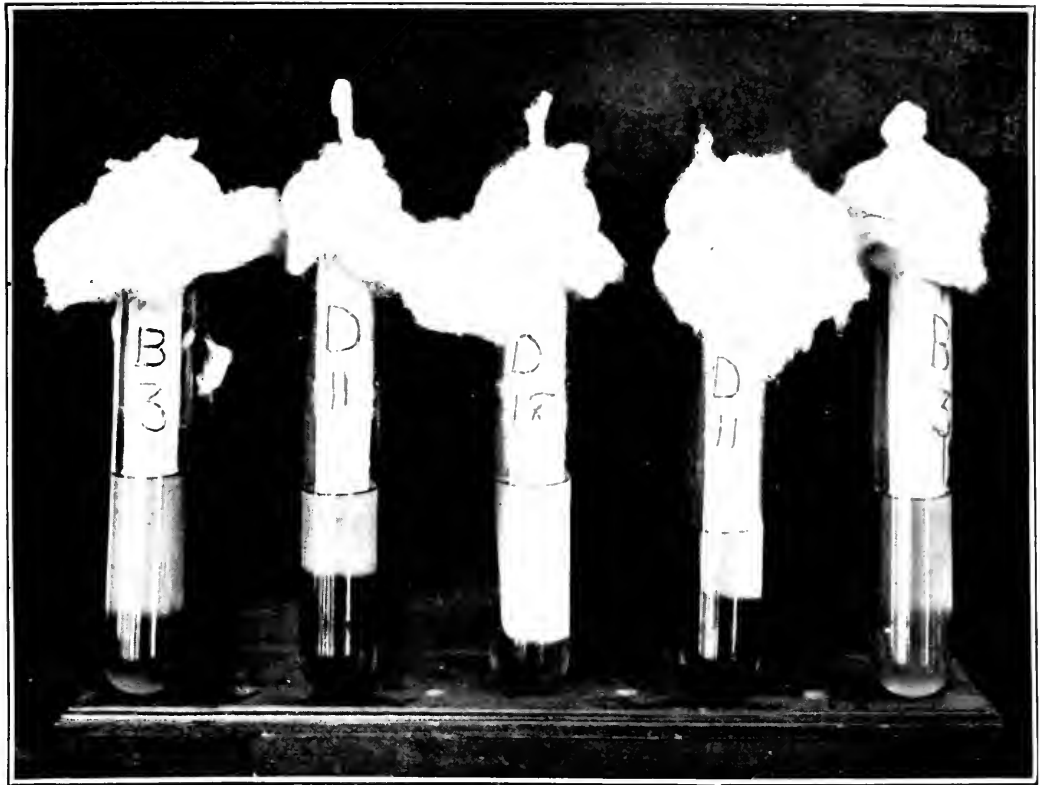


Fig. 3.

74.



A B
Fig. 2.

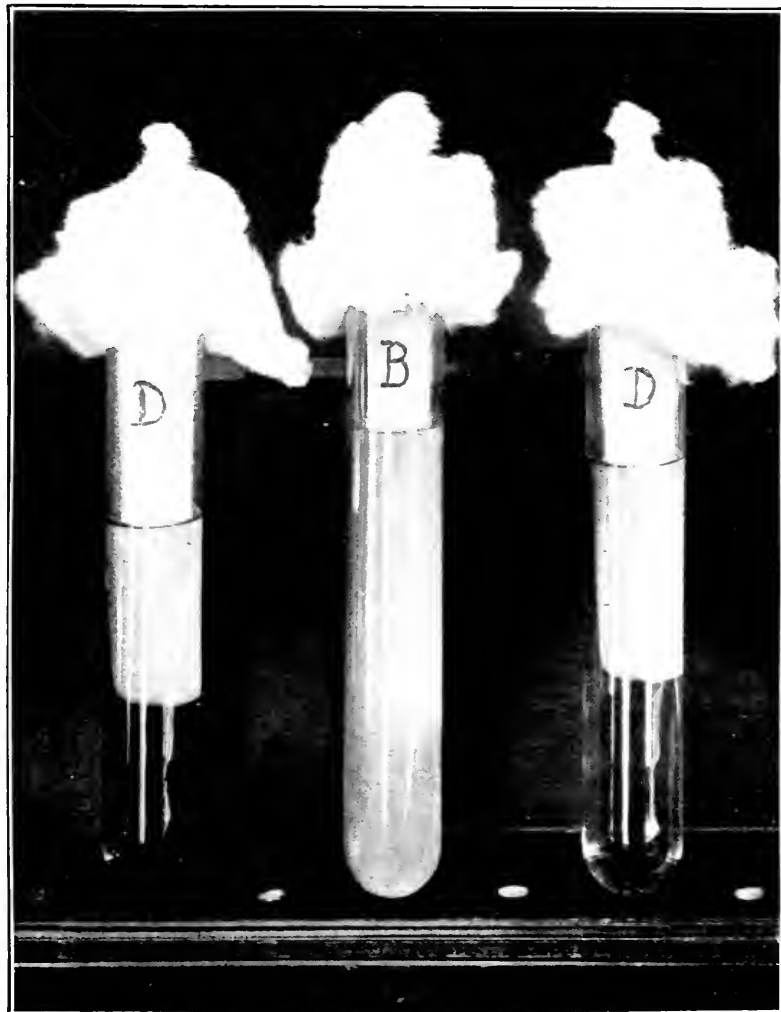


Fig. 4.

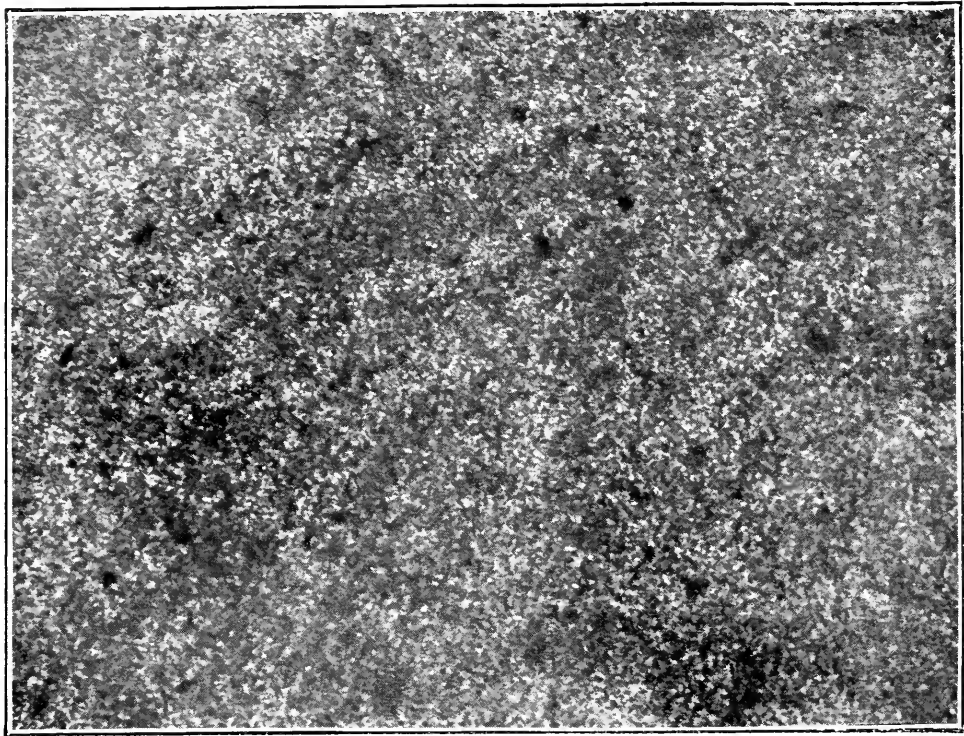


Fig. 1.

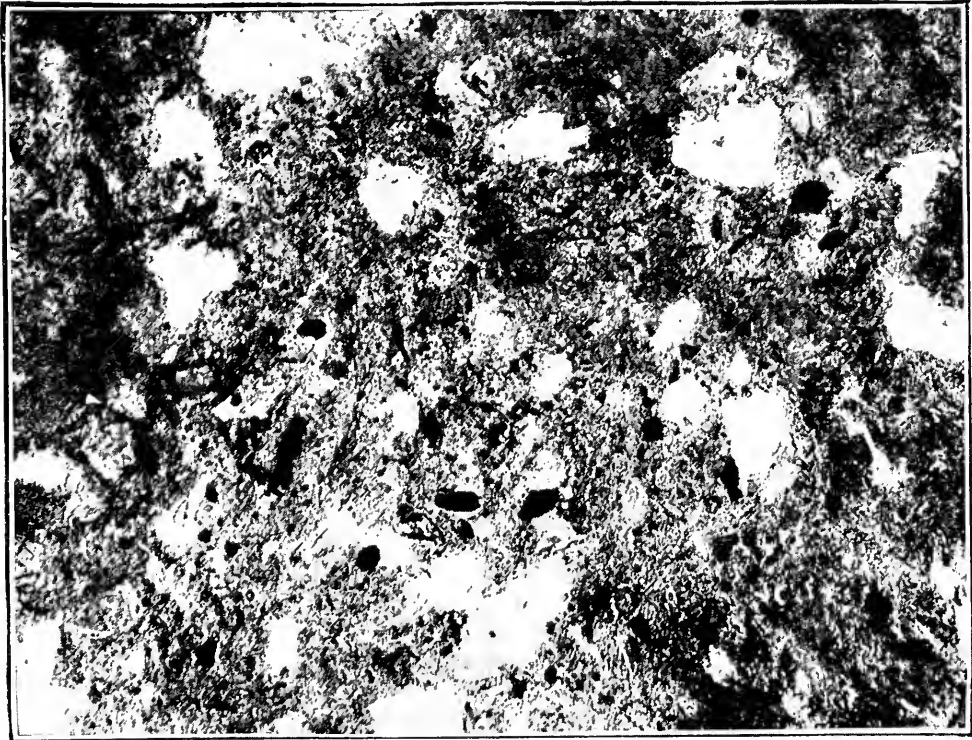


Fig. 3.

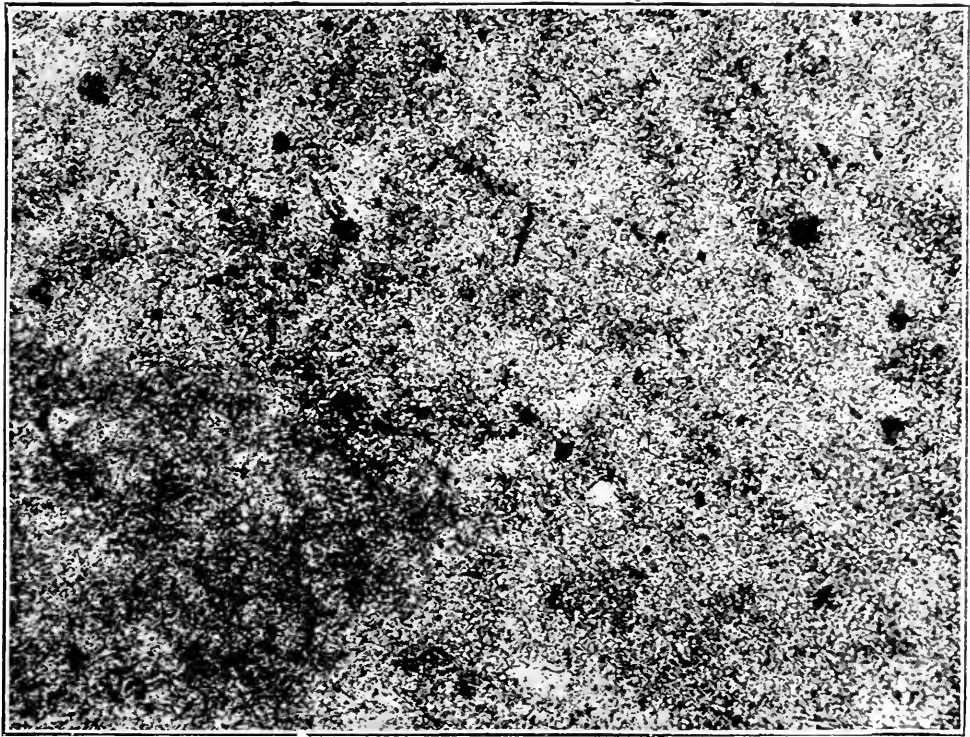


Fig. 2.

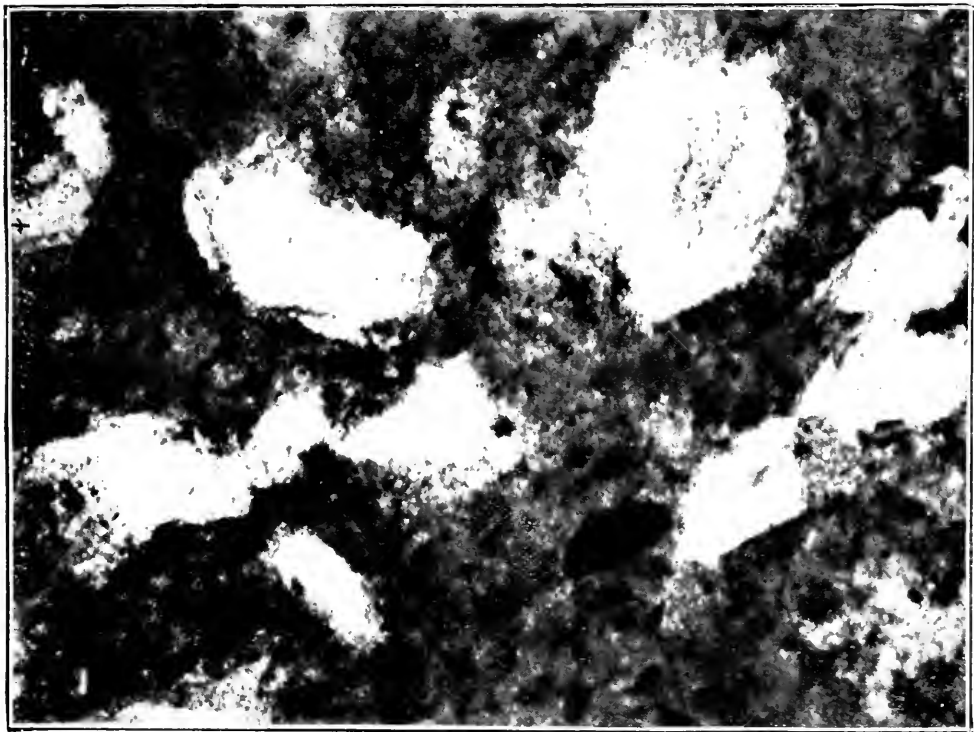


Fig. 4.

CULTIVATION OF THE TRYPANOSOME FOUND IN THE BLOOD OF THE GOLD-FISH.

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Plate III.

SINCE 1903 when McNeal and Novy first recorded that they had obtained cultures of *T. lewisi*, other trypanosomes found in the blood of mammals, more especially the pathogenic ones, *T. brucei* and *T. evansi*, have been successfully cultivated. Besides those parasitic in the blood of mammals, trypanosomes found in the blood of birds, of amphibians, and lastly those found in the stomachs of mosquitoes and other insects, have been grown on artificial media; but so far there has been no record of the successful cultivation of any species of fish trypanosome.

Brumpt (1906), after having described the evolution of fish trypanosomes, particularly those of the eel, in *Hemicleipsis*, and their behaviour in other species of leeches—*Callobdella punctata*, *Hirudo troctina*, *Piscicola geometra*—says that in the medium of McNeal and Novy he was unable to obtain any culture, but that in spite of the difference in the constitution of the medium as compared with that of the blood of the eel he observed some perfectly typical trypanosomes eight or ten days after inoculation. From his various experiments he concludes that the morphological changes which he observed in the trypanosomes were not due to any physical characters of the medium in which they developed, but were the result of a vital reaction of the parasites to a special chemical medium in a particular host.

Lebailly (1906) writes that, contrary to Brumpt's experience, he has found profound changes take place in fish trypanosomes "*in vitro*." The conditions of his experiments were not the same as those of Brumpt's, as Lebailly himself allows. In place of making use of the medium of

McNeal and Novy he simply kept films of blood containing trypanosomes between coverslip and slide by sealing round the edges of the coverslip with paraffin wax. By the end of eight or ten days almost all the trypanosomes in the film had changed into "small granular spherical masses each with flagellum feebly moving."

During the months of May, June and July of 1906, acting on the advice of Dr Dean and helped by him and by Dr Ledingham, I succeeded in obtaining cultures in blood-agar tubes of a trypanosome found in the blood of gold-fishes taken from a pond at Queensbury Lodge, Elstree. The pond is about twenty yards long by about fifteen yards broad. It has a muddy bottom, and contains many weeds and decaying leaves. There is a small inlet and a small outlet so that the water in the pond is slowly changing. In this pond the gold-fishes have been allowed to multiply and to feed naturally for many years. Every fish whose blood I examined harboured trypanosomes. As I examined a great many of all sizes from 1 cm. up to 20 cm. long (the caudal fin which was often damaged being omitted in these measurements), I believe that every fish in the pond from 1 cm. in length upwards is infected. On the other hand I was constantly on the outlook for leeches and although on one occasion (28th May) I had the pond dragged, and also carefully examined with the aid of a hand-lens the gills and the surface of the body between the scales of 178 fishes, I never succeeded in finding a leech. These two facts taken in conjunction—the failure to find leeches during the months of May, June and July, and the constant presence of trypanosomes in every fish examined from 1 cm. in length upwards—suggest that in addition to the known mode of transmission by the leech, there may possibly be some other way or ways by which fish may become infected with trypanosomes.

*Description of the trypanosome as it is found in the
blood of the gold-fish.*

This gold-fish is a species of carp, and the trypanosome found in it (Plate III, Figs. 1 and 2) is, so far as can be judged from its morphology, identical with *T. danilewskyi*, as described by Laveran and Mesnil (1904). As seen in films fixed by methyl alcohol and stained by Giemsa, the body measures $35-45\mu$ long and about 3μ broad; the free flagellum is $15-17\mu$ long; the posterior end of the body is pointed; the kinetonucleus¹, situated about 2.5μ from the posterior extremity, is prominent,

¹ The nomenclature here adopted is that suggested by Woodcock (1906). The smaller body is nuclear in nature, but the term micro-nucleus has already been appropriated for

in dried specimens frequently seen projecting from one side of the body like a short horn: the undulating membrane is large and well folded: the tropho-nucleus is elongated, being about 4μ long by about 2μ broad, and lies somewhat nearer to the anterior than to the posterior end of the body: in addition to the tropho- and kineto-nuclei, the body protoplasm contains chromatic granules varying in number and in size.

Cultivation.

The culture medium found successful was that recommended by McNeal and Novy for the culture of *T. brucei*, slightly modified by Nocht and Mayer, viz.,

I. Extract of 125 grammes of ox flesh in 1000 grammes of water.

Agar	25 grammes.
Peptone	20 grammes.
Chloride of Sodium	5 grammes.
Normal carbonate of Soda solution	10 c.c.

II. Defibrinated rabbit's blood.

One volume of I while still fluid at a temperature of 55° to 60° C. is mixed with two volumes of II in test tubes and the mixture allowed to set in the tube kept sloped in the ordinary way. After the medium has set the tubes are placed upright, capped, and incubated for 24 hours to ensure that the contents are sterile.

Tubes prepared as above were inoculated with blood drawn aseptically from the heart of an infected fish immediately after it had been killed, and were kept in a cool chamber at a temperature of about 15° C. With this medium and under these conditions the proportion of successes was one in three.

The chief difficulties encountered were the technical ones of obtaining blood from the infected fish in an aseptic condition and in transferring this blood to the culture tubes. The heart in these small fishes is very friable and the blood is very coagulable. Capillary pipettes were used to withdraw the blood from the heart. In introducing the point of the pipette great care had to be taken to avoid tearing through the heart wall at the point of fixation. The next difficulty arose from the coagulability of the blood. A little citrate of soda solution (1%) was

the generative nucleus of the Ciliata. Several observers have drawn attention to the fact that the flagellum arises from a small basal granule distinct and separate from the smaller or kinetic nucleus. Professor Minchin referring to these points during the course of his lectures at the Lister Institute defined the blepharoplast as an achromatic body, or centrosome in relation to the flagellum; and was of opinion that the term blepharoplast so often used for the kinetic nucleus should be confined to this basal granule. Whenever the term "Blepharoplast" is used in this paper, it refers strictly to this basal granule.

first drawn into the tube; but even then no time had to be lost in transferring the drawn blood to the culture tube.

Results with rabbit-blood-agar medium.

In tubes that gave successful cultures, developmental forms were first seen on the 6th and 7th days after inoculation, though very probably developmental changes had begun earlier than this. Both living and stained preparations were examined at different stages of the culture. Of living preparations it will suffice to say that small free actively swimming forms were seen at all stages of the culture. These could pass quickly across the field of a 1/12th objective. Large dividing forms, often with several flagella, though they showed very active flagellar movements, had very limited movements of translation. Drawings of stained preparations from a successful culture at different periods after inoculation are reproduced on Plate III, and from these morphological changes can more easily be studied.

Description of stained preparations.

The slides from which these drawings were made were prepared in the following way: a drop taken from the culture tube was spread as a film on a slide, fixed in methyl alcohol, treated with fresh blood serum in the way recommended by Leishman (1904) for sections of tissues to be stained by Romanowsky stains, stained by Giemsa (4 drops to 1 c.c. distilled water for 5 hours), then washed, dried and mounted.

1. *Seventh day of the culture.* Plate III, Figs. 3, to 11, are from a film prepared from culture on the 7th day after inoculation. Fig. 3 represents one of two apparently unaltered forms found in this film. Figs. 4 to 11 have been selected to show how the various cultural forms have been derived from the original trypanosome form as found in the blood of the fish. The initial change seems to consist in a concentration of the endoplasm and a bringing together of endoplasmic structures. The posterior two-thirds of the animal becomes greatly altered in shape and in arrangement of parts. It becomes short and swollen, while the anterior third remains much as it was, so that the whole animal assumes a somewhat tadpole-like appearance as seen in Fig. 4. The kineto-nucleus and the tropho-nucleus, in place of being separated from each other by nearly half the length of the animal, now lie side by side; and the undulating membrane is correspondingly shortened. The tropho-nucleus has become swollen and loose in

structure and its chromatin broken up into chromidia. At this stage apparently division can take place, and Fig. 5 shows division almost completed. The thick swollen end at this stage stains by Giemsa a deep purple. The anterior third unaltered in external form takes no part in the division. The product of division—resembling *Crithidia*—as seen still attached in Fig. 5, and free in Fig. 6, has apparently the power of multiplying freely. This is illustrated by Figs. 7, 8 and 9. Figs. 8 and 9 are probably agglutinations, but the point now is that in this *Crithidia*-like form free multiplication has taken place. Fig. 10 shows a form where the body has become elongated, but where the kineto- and tropho-nuclei are still close together. In Fig. 11 the kineto-nucleus has come to lie at a considerable distance behind the tropho-nucleus. As will be seen below, trypanosome-like forms (with body elongated and kineto-nucleus well behind the tropho-nucleus) become more numerous at later stages of the culture.

2. *Twenty-first day of the culture.* Figs. 12 to 15 inclusive are from a film prepared from a drop taken on the 21st day of the culture. The *Crithidia*-like form (Figs. 12 and 14) is still in evidence. Along with this are other forms with well developed undulating membrane (Fig. 15), and others where the kineto-nucleus is anterior to the tropho-nucleus and where division is going on (Fig. 13).

3. *The twenty-eighth day of the culture.* Plate III, Figs. 16 to 26, are from a film taken on the 28th day of the culture. The preponderance of bulky forms, the signs of considerable nuclear activity, and the presence of so many coarse granules in the body protoplasm are here very striking. The granular condition of the protoplasm may be due to some influence of medium (most probably the result of some accidental change that had taken place in it about this time) and may possibly be analogous to the granular condition of mast cells. Mast cells, as is known, are found in the mesentery more abundantly than in other parts of the body; and in it in greater numbers during digestion or after a meal. Forms with the kineto-nucleus close by the side of the tropho-nucleus—an arrangement seemingly favourable to free multiplication—are still found (Figs. 17 to 20 and Fig. 23). Fig. 16 shows multiple fission. Other forms more trypanosome-like than any hitherto seen in the culture now appear (Figs. 21 and 24). In Figs. 18, 23 and 25 the tropho-nucleus has divided before the kineto-nucleus, while in Figs. 21 and 24 the reverse is the case. In Fig. 21 it is difficult to see how the smaller of the dividing portions was to get its tropho-nucleus; but I have observed an exactly similar condition in a blood film from a rat that had

been inoculated with *T. lewisi*, so that such apparently anomalous forms are not confined to culture tubes. In Fig. 24 the true blepharoplasts or basal granules, from which the flagella arise distinct from the kineto-nuclei, are clearly seen.

4. *Forty-third day of the culture.* Figs. 27 to 35 are from a film taken on the 43rd day of the culture. In all, the body protoplasm is free from granules. It would thus appear that the influence or influences, to which was due the granular condition seen in forms from the 28th day of the culture, were of a temporary nature. Forms that are *Crithidia*-like by the relative position of kineto- and tropho-nuclei are here also seen; and multiplication by division is still actively going on. In Fig. 32 the kineto-nucleus is getting behind the tropho-nucleus; while in Figs. 33, 34 and 35 trypanosome-like forms resembling mammalian trypanosomes more closely than adult fish trypanosomes, are represented. These divide by unequal longitudinal division, and Fig. 33 shows division begun with the division of the blepharoplast. This is often seen to be the case in mammalian types. Division of the kineto-nucleus, whether it takes place before or after division of the tropho-nucleus, is probably always preceded by division of the true blepharoplast or basal granule, and commencing formation of a new flagellum. These trypanosome-like forms were found to be more numerous at this period than at earlier stages in the culture.

How long the culture lived after this I do not know. When I returned from a holiday seven weeks later it had died out. Sub-cultures that I inoculated just before leaving also showed no sign of life on my return.

SUMMARY AND CONCLUSIONS.

1. The trypanosome of the gold-fish has been successfully cultivated on the medium of McNeal and Novy.

2. Preparatory to division in culture, the original trypanosome as found in the blood of the fish assumes a somewhat tadpole-like appearance, the endoplasm and its contained structures being collected together in the swollen posterior end. The kineto-nucleus now lies close to, and alongside of, the tropho-nucleus, and the latter has become swollen and loose in structure with its chromatin broken up into chromidia. The anterior third or more of the trypanosome undergoes little or no change in form and does not take part in division. It is thus easily seen how the product of the preliminary division comes to have a *Crithidia*-like appearance.

3. The product of this preliminary division—*Crithidia*-like by the relative position of kineto- and tropho-nuclei—is capable of freely multiplying.

According to Brumpt it is in the *Crithidia*-like form that free multiplication of the eel trypanosome first takes place in the stomach of *Hemiclepsis*. It may well be that in this case also the *Crithidia*-like form is arrived at by steps such as are here figured and described.

4. *Crithidia*-like forms are found at all stages of the culture and along with them at various stages other forms where the body is elongated and the kineto-nucleus still close to the tropho-nucleus, and yet other forms where the kineto-nucleus lies at varying distances behind the tropho-nucleus until true trypanosome-like forms are reached.

5. These trypanosome-like forms (resembling mammalian trypanosomes rather than those of the fish from which they are derived) are most numerous in the later stages of the culture.

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DESCRIPTION OF PLATE III.

Figs. 1 and 2. Trypanosomes from film of blood of gold-fish.

Figs. 3—11. Forms found in film prepared from culture on the 7th day after inoculation.

Fig. 3, unaltered form. Fig. 4, form showing the change that takes place preparatory to division. Fig. 5, primary division almost completed. The anterior part of the trypanosome which retains its original form takes no part in division, and the product of division is *Crithidia*-like in appearance. Figs. 6—9, *Crithidia*-like forms that multiply freely. Fig. 10, form with body elongated but with kineto-nucleus still close to (here somewhat anterior to) the tropho-nucleus. Fig. 11, form where the kineto-nucleus has come to lie posterior to the tropho-nucleus.

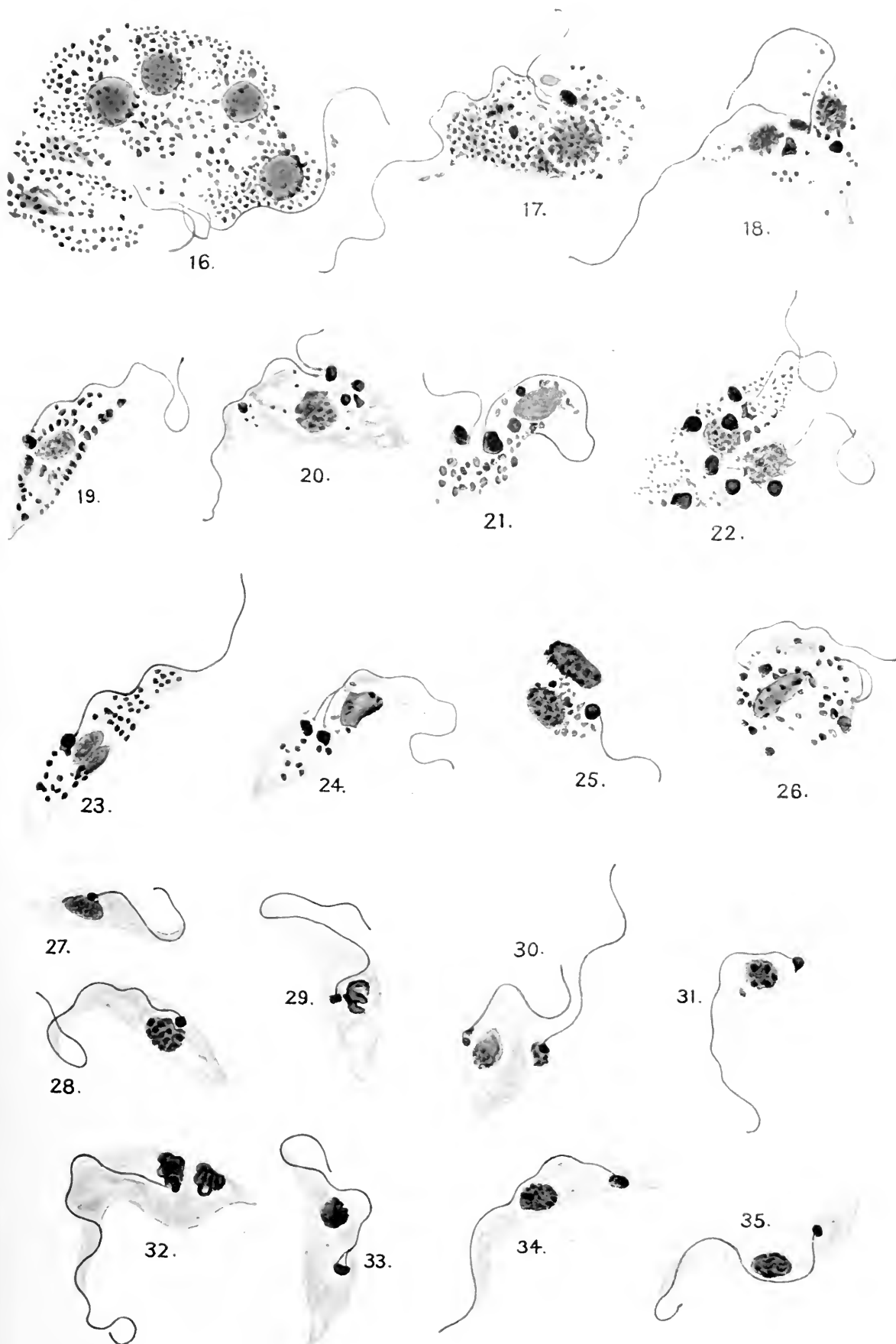
Figs. 12—15. Forms from a film prepared from cultures on the 21st day after inoculation. Figs. 12 and 14, *Crithidia*-like forms. Fig. 13, dividing form with kineto-nucleus anterior to the tropho-nucleus. Fig. 15, form with well-developed undulating membrane.

Figs. 16—20. Forms from film prepared from culture on the 28th day after inoculation. The bulky form of some, the number of dividing forms and the coarse granular appearance of all are conspicuous. In Figs. 18, 23 and 25 the tropho-nucleus has divided before the kineto-nucleus : in Figs. 21 and 24 the reverse is the case : while in Figs. 17 and 20 the true blepharoplasts are the first to divide. Fig. 24 shows clearly the origin of the flagella from small basal granules, or true blepharoplasts, distinct from the kineto-nuclei.

Figs. 25—35. Forms from film prepared from culture on the 43rd day after inoculation. Note the absence of granules in the protoplasm, the *Crithidia*-like forms, dividing forms, and trypanosome-like forms—the latter being more numerous than at earlier stages of the culture.

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THE EXAMINATION OF SOME COMMERCIAL CARBOLIC ACIDS AND DISINFECTING POWDERS.

By M. WYNTER BLYTH, B.A. (Cantab), B.Sc. (Lond.), F.I.C.

THE bacteriological testing of disinfectant powders is a subject which has received very little attention. In comparing the relative values of powders it is not sufficient to make a watery solution of the powder and filter or allow to stand, for in this way only the value of those substances which are completely soluble in water is obtained, while those more or less insoluble oils, which have a high germicidal value when brought in contact with micro-organisms either in the form of an emulsion or in a state of very fine division as in powder, are prevented from coming in contact with the organism. A solution of the powder in water has been used by many people in this country, and has also been used by Robertson and Severn (1904—1905) in Cape Colony. These authors fully recognised that such a method was unsatisfactory, for they say, "The objections to this method as giving any clue to the operations of powders in practice are many and obvious, but it is difficult to imagine a fairer method of procedure."

The method they employed was as follows:—

"One hundred grammes of the powder are thrown into a litre stoppered measure, made up to the mark with sterile distilled water shaken at frequent intervals for four hours, and then left to subside till the same hour next day at which the water was added. The supernatant fluid is syphoned off and 10 c.c. of it regarded as equal to one gramme of the powder."

The function of a disinfectant powder, as I understand it, is to disinfect the surface of more or less solid infected matter. It is essentially a surface disinfectant. If a disinfectant be required to penetrate into the body of a substance either a liquid disinfectant should be chosen or possibly a powder containing a readily soluble substance such as phenol.

The action of the two classes of powders, soluble and insoluble, is obviously quite different, and according to the purpose for which the powder is to be used so one or the other should be chosen. The chemical and bacteriological examination of all powders is of the highest importance owing to the large amount used each year and the enormous amount of cheap and worthless powder which is sold as carbolic powder, or as equivalent in germicidal value to some stated quantity of carbolic acid. It is necessary to take as our standard, when testing powders, a carbolic acid powder containing the same quantity of oil per cent. as the one we are testing. For instance, if a powder A, containing 10% of an oil, kills the organism with a dilution of 1:200, and a 10% pure carbolic acid powder kills the organism with a dilution of 1:10, it is obvious that our powder A is 20 times more effective than is the same quantity of carbolic acid *placed under the same conditions*. It is not possible to keep a standard carbolic acid powder for comparison—the powder must be made up fresh for each determination.

Messrs Calvert and Co. of Manchester very kindly supplied me with various powders and acids of definite composition. Their powder marked A, composed of 10% pure phenol mixed with a silica base, gave with a particular organism when fresh a dilution figure of 1:130; after the bottle (of clear glass) had been standing in the light for some weeks it gave a dilution figure of 1:150, and when last tested a dilution figure of 1:160; freshly distilled phenol in distilled water giving a dilution figure of 1:110 on each occasion with the same organism. Further, the phenol when extracted by repeated exhaustion with petroleum ether gave at first a beautifully crystalline mass of phenol; when last extracted it was in the form of a yellow oil which refused to crystallize. Again, a 1% solution of phenol was prepared and divided into two parts, one being placed in a tightly corked yellow bottle and kept in the dark, the other in a clear bottle and kept in the light. The following results were obtained:—

	Dilution figure	Fresh solution
Phenol solution fresh	1 : 110	
Kept in the dark for 1 month	1 : 130	1 : 110
In light ,, 1 ,,	1 : 130	1 : 110
Kept in dark ,, 2 months	1 : 130	1 : 110
In light ,, 2 ,,	1 : 150	1 : 110

In a previous paper attention was called to the increase in germicidal value of cresylic acids on standing in the light, and it was then said that it was possible that the same thing might happen with phenol. From the above experiments and many others it is evident that in any experiments on germicidal value, taking phenol as a

standard, it is absolutely necessary to take a freshly distilled phenol and to make up the solution immediately before use, unless it is proved by repeated experiments that the phenol solution gives the same results over a long period of time. In testing powders I take silica as the base and add to this the same quantity of freshly distilled phenol as there is oil in the sample to be tested; for instance, in testing a 10% carbolic powder (cresylic powder), the standard is 90 grammes of dry silica and 10 grammes of pure phenol; for a 15% powder the standard is 85 grammes silica and 15 grammes of phenol, and so on.

The increase in germicidal value observed in old samples of commercial Carbolic Acids, in powders, and indeed in comparatively pure ortho, meta- and para-cresol and in phenol, makes it impossible to say from a direct estimation of germicidal value how much cresylic or carbolic acid the acid or powder contains. To do this it is necessary to make both a chemical and a bacteriological estimation.

The direct estimation of phenol in the presence of the cresols is a matter of great difficulty. The ordinary method of estimating it by fractional distillation is far from accurate, giving only approximate figures. The methods given in various text books for drying with lead oxide, sodium acetate, etc. are all unsatisfactory, and after a prolonged trial I have abandoned them as useless. It is impossible to get an accurate estimation by extracting with dry volatile solvents, as I find the phenol cannot be freed from the solvent without loss even with the greatest care and working at a low temperature. The only method which I have found applicable to powders containing *only* phenol is to extract with water and estimate the phenol in the water solution by means of bromine. With powders containing small quantities of phenol, the total oil may be estimated with accuracy by extracting with petroleum ether boiling at 40° C., and distilling off the bulk of the ether at a temperature not exceeding 80° C., then allowing the oil to stand for 24 hours over sulphuric acid in a large desiccator. With blast furnace oils and coke oven oils, such as izal, the experiments are not altogether satisfactory, as the following show :

100 grammes powder 10 % izal oil extracted with pure pentane and allowed to stand at ordinary temperature for 24 hours :

Weight of oil found	Theoretical	Loss
9.79 grammes	10.0	2.1 %
After 3 days weight of oil found		
9.43 grammes	10.0	5.7 %

Izal powder extracted with pure pentane and then mixed with oxide of lead :

	Grammes found	Theoretical	Loss
24 hours	9.88	10.0	1.2 %
3 days	9.55	10.0	4.5 %

These results were confirmed by taking definite weights of izal oil kindly sent to me by Messrs Newton, Chambers and Co. It was found impossible to estimate the oil within 1% by the use of any volatile solvent, and direct distillation was found even more unsatisfactory. These methods are only accurate enough to tell us whether we are dealing with a 10%, 15% or other approximate quantity of oil in such a substance as Izal Powder.

The estimation of water and phenol in commercial carbolic acids and the phenol in carbolic powders may be carried out as follows:— at least 100 grammes of the powder, if it contains as little as 10% of oil, are extracted with petroleum ether, the ether driven off and the total oil weighed. If the powder contains slime or soap, or anything that combines with the acids, sulphuric acid is added before extracting with the ether. For distillation I use a short condenser, filled at first with cold water, but through which water is not kept running. If the oil or powder contains less than 10% of phenol it will only be necessary to distil one-third of the bulk, if more, it may be necessary to distil a further quantity, and in some cases the distillation must be carried to dryness. The distillate is weighed, then well mixed and divided into two parts, to one of which lumps of freshly ignited calcium chloride are added, and both are tightly corked and put in a dark cupboard for at least 12 hours. The germicidal value of both portions of the distillate is then determined as described in *The Analyst*, May 1907, and compared with freshly distilled phenol and ortho-cresol.

The use of calcium chloride in this way enables us to dispense with the use of milk, for in the first place we are determining water cresol and phenol, and in the second cresol and phenol. In the case of good powders extracted by petroleum ether, the use of calcium chloride is unnecessary. With carbolic acids however there is always a little water.

Example 1. Carbolic acid C, weight taken 28.44 grammes, distillate 11.319 grammes.

	Distillate with water	Distillate without water
Dilution figures	1 : 260	1 : 270
	With cresol	Phenol
Controls	1 : 270	1 : 130

The sample is free from phenol, but contains water; the quantity in the distillate being such that a dilution of 1:260 is equivalent to 1:270, in other words our dilution of 1:260 is really a dilution of $\frac{260}{270}$ in 260, and every 1 gramme of distillate contains only .963 gramme of oil and .037 gramme of water.

Our whole distillate contains $11.319 \times .037$ grammes water, and this is obtained from 28.44 grammes of our sample, therefore the sample contains $\frac{11.319 \times 3.7}{28.44}$ grammes of water.

Example 2. 50 grammes of a carbolic acid guaranteed to contain 10 % of real phenol. Distilled 25 grammes.

	With water	Without water D_2
Distillate	1 : 250	1 : 260
	Cresol D_1	Phenol D_2
Controls	1 : 270	1 : 130

Then percentage of phenol in the water-free distillate

$$\frac{100 (D_1 - D_2)}{D_1 - D_2} = \frac{100 (270 - 260)}{270 - 130} = 7.14 \text{ grammes.}$$

Total oil in distillate = $\frac{25}{25} \times 25 = 24.04$ grammes.

Total water in distillate = .96 gramme.

This is derived from 50 grammes of the sample, therefore the percentage of water is 1.92. The total phenol in the distillate is $\frac{7.14 \times 24.04}{100} = 1.72$, and this quantity is contained in 50 grammes of

the sample, therefore the sample contains 3.44 % of phenol. All samples of carbolic acids and carbolic powders which contain only phenol or crude carbolic acid and cresol may be examined in this way. The germicidal value of such samples, together with the temperature at which the fractions distil over, their specific gravity, solubility in alkali, and the amount of tarry matter left behind in the still, gives us all the information necessary with regard to these powders and acids.

With powders made with blast furnace oil, coke oven oils, and other coal tar distillates, which either contain very little carbolic or cresylic acids, or none, the question arises as to their value as germicides under the conditions of some standard test, as compared with carbolic acid. This value is one which it is also necessary to ascertain in the case of commercial carbolic acid powders.

Method for testing the germicidal value of disinfecting powders against naked organisms.

Organism. Twenty-four hours' old culture of *B. typhosus*.

Broth. Reaction + 15 to phenol-phthalein. Composition, Liebig's extract of meat 20 grammes, peptone (Witte's) 20 grammes, sodium chloride 10 grammes, tap water to make 1000 c.c.

Temperature of experiments, 17° C. exactly (this refers to the actual temperature of the tubes and not to the room temperature).

Time of comparison, 10 to 12½ minutes. (All apparatus and distilled water and sterilised before use.)

1. Estimate the total quantity of oil present in the sample to be tested, and weigh out a corresponding quantity of silica base and pure freshly distilled phenol.

For example, 9 grammes silica, 1 gramme phenol made up to 100 c.c. with distilled water = 10% solution of a 10% powder.

The powder and water are shaken vigorously together and poured out in small quantities at a time (shaking at intervals) into a narrow measuring glass graduated into one-tenths of a c.c.; the required quantity of distilled water is added to bring the strength down to the dilution required and the various mixtures shaken and poured into glass stoppered tubes. It is best to calculate the dilution on the actual phenol present. This way of making dilutions is only possible when we have a soluble substance such as phenol.

2. Various quantities of the powder to be tested are now weighed out into glass stoppered tubes, which if we have a very active oil present must have a capacity of at least 50 or 100 c.c., and the requisite amount of distilled water run into each tube to bring the mixture up to the dilution required. Each gramme of powder being taken as occupying 1 c.c., as in the case of the control. The tubes are placed in a small tank containing water at 17° C. The bottom and sides of the tank are covered with felt, a thermometer being fitted by means of a perforated cork through one of the sides. The top of the tank is of thin copper in the form of a tray partly covered at one end to prevent the tubes falling out (or a rubber band may be passed round the tank to keep the tubes in position). The tank is so fixed that it may be rocked either by hand or foot or by some mechanical means. The rocking must be done so that the tubes are completely and gently turned right on end once during a period of at least every three seconds, so as to ensure the powders being kept in intimate contact with the organism during the

whole of the time. The actual speed of the rocking, providing it is kept within reasonable limits, has little, if any, effect on the results obtained.

The tubes are inoculated by allowing five drops of broth culture for every 5 c.c. of powder and liquid present in each tube. The tubes can be taken out and replaced without stopping the rocking. Inoculations are made into broth in the ordinary way at intervals of $2\frac{1}{2}$ minutes up to 15 minutes, and the dilution figure is taken as that which gives a + value at the end of 10 minutes and a - value at the end of $12\frac{1}{2}$ minutes. The process may be simplified by taking inoculations at the end of 10 minutes, or any other agreed definite limit. In the following example the dilution figures refer to the actual amount of phenol, or any other oil present in the dilution.

I. A sample of carbolic acid A, specially prepared for this research by Messrs Calvert and Co., containing 10% crystal carbolic acid fusing at 40° C. silica base.

Dilution	Time in minutes					
	$2\frac{1}{2}$	5	$7\frac{1}{2}$	10	$12\frac{1}{2}$	15
1 : 220	+	+	-	-	-	-
1 : 230	+	+	+	+	-	-
1 : 240	+	+	+	+	+	+
Fresh phenol and silica base control :						
1 : 180	+	+	-	-	-	-
1 : 190	+	+	+	+	-	-

The 10% carbolic A is equivalent to $10 \times \frac{230}{190}$ or a 12% pure carbolic powder when freshly prepared.

The above carbolic powder, with an organism of the coli group, gave the following values:—

- | | | | | | |
|---|-----|-----|-----|-----|---------|
| (1) Shaken dilution figure | ... | ... | ... | ... | 1 : 130 |
| (2) Not shaken | ... | ... | ... | ... | 1 : 130 |
| (3) Fresh distilled carbolic acid and silica base shaken | ... | | | | 1 : 120 |
| (4) Ordinary fresh distilled phenol in water without silica | | | | | 1 : 100 |

The influence of shaking and the change that takes place on standing are not so marked with the more resistant *B. coli* as it is with the more delicate *B. typhosus*.

II. A sample of silica base powder containing 7% liquid carbolic acid (practically pure cresylic acid) and 3% crystal carbolic acid fusing at 40° C. specially prepared by Messrs Calvert and Co. (Note the

3% carbolic acid was readily detected by extraction and dilution as already described.)

(a) Dilution figure found ... 1:460

(b) Phenol and silica dilution figure ... 1:200

The 10% powder is equivalent to $10 \times \frac{4.6}{20} = 23\%$ pure carbolic acid powder.

III. Experiments on a powder containing an insoluble oil. Samples of 10% and 15% izal powder were bought in the open market (these powders are commercially known as No. 1 and No. 2). The exact quantities of oil present in each sample could only be determined, as already described, approximately. The control powders were made up with 10% fresh phenol for the 10% izal powder and 15% fresh phenol for the 15% izal powder. The powders were weighed out, the water added, and the experiments proceeded with at once; this is important, as somewhat higher results will be obtained if the water and powder be left in contact for some time (24 hours).

	Dilution	Time in minutes					
		2½	5	7½	10	12½	15
15% izal powder	{ 1 : 1300	+	+	+	-	-	-
(The dilutions refer to the actual quantity of oil)	{ 1 : 1500	+	+	+	+	-	-
	{ 1 : 1700	+	+	+	+	+	-
Fresh phenol and silica control	{ 1 : 190	+	+	-	-	-	-
	{ 1 : 200	+	+	+	+	-	-

The 15% izal powder is equivalent to $15 \times \frac{15.00}{200} = 112\%$ pure carbolic acid.

In the same way the 10% powder was found to be equivalent to a 70% pure carbolic acid powder.

A large number of experiments were made on an organism of the *B. coli* group, with the powder shaken as in the method described above and with the extracted oil, which was then saponified by resin and soft soap. The result being that for this particular organism the same coefficient was obtained for the oil both when shaken in powder form and when saponified. Showing that when powders are well mixed, and are kept in intimate contact with the organism, they act in the same way as emulsions. When powders of this class, containing more or less insoluble oils, are not shaken, as in the method of Robertson and Severn, very different results are obtained, and results which I do not think can in any way represent the action of the oil when actually brought in contact with the organisms, depending as they do simply on the solubility of the oil. This solubility being far from desirable in a

powder except for special purposes, when a powder containing carbolic acid should be chosen.

Organic matter, such as faeces-emulsion, milk, etc., may be introduced into this test in exactly the same manner as it can be introduced into the ordinary test for liquid disinfectants, and the introduction of organic matter is in my opinion quite as essential in testing the value of disinfectant powders as it is with liquid disinfectants.

This research deals only with those powders containing silica base. The influence of various other bases, such as Kieselguhr, lime, road sweepings, destructor refuse and other substances, on the germicidal value of powders is being studied at present, and I hope to report the results in a future paper.

Summary and conclusions.

1. The phenol solutions used as a standard in the bacteriological testing of disinfectants should be made from pure freshly distilled phenol.

2. The actual quantity of phenol, cresylic acid and water present in commercial carbolic acids and in carbolic powders may be estimated by extracting the oils and testing their germicidal power.

3. The comparative germicidal value of disinfectant powders may be estimated by keeping the powder and organism in contact by mechanical means during the whole period of the experiment.

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AN INVESTIGATION OF THE LAWS OF DISINFECTION.

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INTRODUCTION.

THE work of Pasteur, showing the action of preservatives to be due to their toxic effect upon micro-organisms, and the extension of the same explanation, by the work of Lister, to cover the case of disinfectants and disease germs, was followed by a considerable amount of successful work dealing with the disinfectant action of vast numbers of substances upon putrefying matter. (Buchholtz 1875 ; Jalan de la Croix 1881.)

Robert Koch (1886), however, is responsible for the first systematic experiments on disinfection, using pure cultures of bacteria. By means of his "thread" method he investigated the effect on anthrax spores of the then popular disinfectants, carbolic acid and sulphur dioxide, and of many substances previously uninvestigated. He confirmed previous work, demonstrating the powerful disinfectant, and even greater antiseptic, properties of mercuric chloride, and the great reputation of this salt may be said to date from these experiments.

Shortly afterwards much interest was excited by the appearance of the first of many emulsified disinfectants, creolin, and this substance was investigated by many contributors to the study of disinfection, *e.g.*, Esmarch (1887) working with putrefying liquids, and Henle (1889), who worked with cholera and typhoid. The latter also showed that creolin contained higher homologues of phenol (cresols, etc.) which, themselves only slightly soluble in water, are conveniently emulsified by the addition of soap; these higher phenols are at the same time more powerful disinfectants and less poisonous than carbolic acid. Fraenkel (1889) showed that the extra disinfecting power of crude over pure carbolic acid was due also to the admixture of higher phenols; he

investigated these compounds in pure samples and demonstrated their excellent disinfecting properties by means of experiments with anthrax spores.

Geppert (1889 and 1891) published experiments which showed that the disinfecting powers of mercuric chloride had been over-estimated, and criticised previous work and especially Koch's "thread" method, in view of the unavoidable carrying-over of traces of sublimate from the disinfecting fluid into the test cultures, growth being thus inhibited, although the organisms might not have been destroyed at the time of sub-culture. He proved this by adding to his test culture enough ammonium sulphide to precipitate the mercuric chloride carried over, and he worked directly with emulsions of bacteria in place of the silk threads soaked in broth cultures.

Behring (1890) published the results of a number of experiments upon disinfection and vigorously combated Geppert's views in respect of mercuric chloride, but Koch's "thread" method has since been largely abandoned for the above reason.

In 1897 Krönig and Paul published their classic work upon disinfection; their "garnet" method will be referred to later, and by its means they studied most carefully the disinfecting process, using definite species of organisms and disinfectants of every class in widely varying concentration. Their experiments were conducted with the utmost care, and the valuable quantitative results which they obtained form the most important contribution to the subject which has yet appeared.

The more recent work on disinfection has been concerned chiefly with the standardisation and comparison of different disinfectants from a practical point of view. Rideal and Walker (1903) described a method by means of which the value of disinfectants could be expressed as a numerical ratio, using pure phenol as a standard. Their method, with certain modifications, was used in the present investigation and was briefly as follows:—a definite small amount of broth cultures of the same species and age was added to a constant volume of disinfectant solution. By making a series of trials with varying concentrations of the disinfectant to be tested, and of pure phenol under otherwise similar conditions, they determined the relative concentrations of the two fluids required to bring germicidal action to completion in the same time. The ratio of the reciprocals of such concentrations was taken to express the disinfecting value of the unknown disinfectant in terms of carbolic acid as a standard. This ratio was called by them the 'carbolic acid coefficient.'

Madsen and Nyman (1907), using Krönig and Paul's method, have further investigated the progress of the reaction during the disinfection of anthrax spores by mercuric chloride and heat. Their experiments are in many ways parallel to my own and their results will be discussed later.

Three classes of disinfectants have been investigated in the present research.

- (1) Dissolved organic disinfectant:—phenol.
- (2) Emulsified disinfectants, containing insoluble coal tar derivatives held in very fine suspension in water by means of soap, glue, etc.:—disinfectant "A."
- (3) Metallic salts:—mercuric chloride and silver nitrate.

SECTION I. REACTION VELOCITY OF DISINFECTION.

Krönig and Paul (1897) published the results of a few careful experiments with anthrax spores, using mercuric chloride of different concentrations. Their method was as follows:—garnets were selected of equal size, carefully cleaned and dipped into an emulsion of sporing anthrax bacilli, which were allowed to dry on their surface in a thin film. The garnets were immersed in the solution of mercuric chloride, and from time to time a definite number was taken, and all traces of sublimate were removed by gentle washing and treatment with ammonium sulphide. These garnets were then well shaken in a measured quantity of water to detach the adherent spores and a constant amount of the washings was plated. The number of germinating organisms was counted. It was shown that by thus shaking in water a fairly constant definite proportion of the total organisms was detached.

From a study of the figures obtained in Krönig and Paul's experiments, it is evident that the disinfection process is a gradual one. No definite conclusions were drawn by the authors themselves, but Ikéda (1897) attempted to explain the course of the reaction and deduced from their results the following formula:—

$$\frac{n_1}{n_2} \times \frac{t_1}{t_2} = \text{constant},$$

where n_1 and n_2 are the numbers of organisms surviving after times t_1 and t_2 respectively.

On plotting the results of Krönig and Paul (see Fig. 1, ordinates representing the numbers of surviving bacteria, and abscissae the corresponding times), the points were found to lie upon fairly smooth

curves. The form of these curves suggested the existence of a logarithmic relation between the two variables, time on the one hand, and the number of surviving bacteria on the other. In fact the curves appeared to be very similar in form to that expressing the course of a so-called "unimolecular reaction," and the equation

$$-\frac{dC}{dt} = KC,$$

or

$$\frac{1}{t_2 - t_1} \log \frac{C_1}{C_2} = K$$

was found to be applicable to the case of disinfection, if, in place of the terms C_1 and C_2 expressing concentration of reacting substance, numbers of surviving bacteria were inserted, thus:—

$$\frac{1}{t_2 - t_1} \log \frac{n_1}{n_2} = K,$$

where n_1 and n_2 are the numbers of bacteria surviving after times t_1 and t_2 respectively.

In their recent work Madsen and Nyman (1907) also discover from Krönig and Paul's figures that, in the disinfection of anthrax spores with mercuric chloride, the reaction proceeds according to the equation given above. A table¹ is given (p. 390) showing the good agreement between the numbers of surviving bacteria actually counted by Krönig and Paul, and those calculated by Madsen and Nyman on the supposition that the above relation holds good. Their own experiments with mercuric chloride and anthrax spores, using Krönig and Paul's methods, have fully confirmed the previous work and have shown that the same law holds good for the destruction of anthrax spores by heat.

It was necessary, first of all, to repeat² these enumeration experiments of Krönig and Paul, using a different germicide, and the following experiments were therefore made with anthrax spores, phenol being substituted for mercuric chloride. In order to obtain perfect comparison with further experiments upon a non-sporing organism (*Bacillus paratyphosus*), for which the garnet method was unsuitable, the experiments were made as follows:

An agar culture of anthrax, about a week old, consisting almost entirely of spores, was emulsified with a little distilled water and centrifugalised for a few

¹ This table will be referred to again later (Section II, p. 130).

² Some enumeration experiments of Spiro and Bruns (1898) with pyrocatechin and anthrax spores roughly confirm those of Krönig and Paul; they were, however, not made with any very great accuracy, but form an interesting confirmation.

minutes to get rid of any scraps of agar or aggregated masses of spores. The supernatant liquid was pipetted off and heated to 80° C. for about five minutes. This formed the emulsion used for the experiments ; it was preserved in the cold room and was found to suffer very little change.

Everything used in the experiments, tubes, pipettes, etc., was previously sterilised, and the disinfectant, in this case phenol (5 %), was measured into a test tube and placed in a water-bath whose temperature was maintained constant, in most experiments at 20° C. A small amount of the emulsion (depending upon the concentration of spores, determined by a previous control experiment) was added

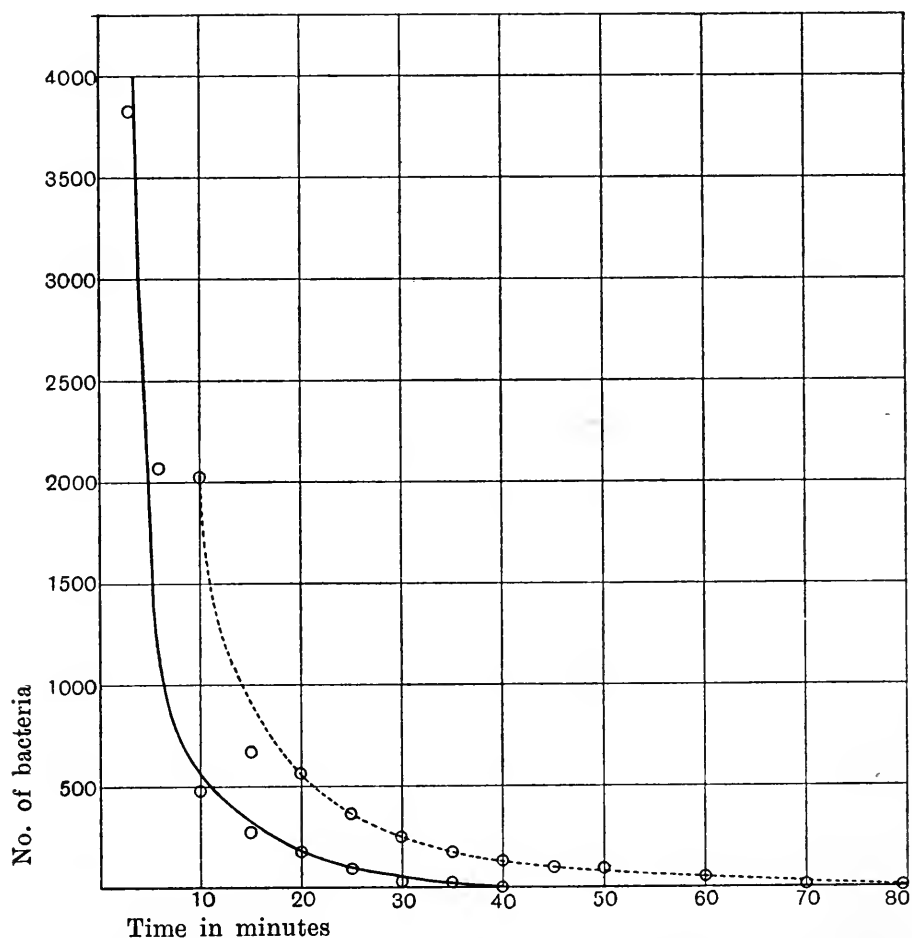


Fig. 1. Illustrating the results of Krönig and Paul's experiments. Continuous curve, disinfection of anthrax spores with 2.1 per 1000 HgCl₂. Dotted curve, disinfection of anthrax spores with 1.1 per 1000 HgCl₂.

to the disinfectant tube. At definite intervals of time samples were withdrawn, diluted, and immediately plated on agar. These samples were obtained by means of a capillary pipette fitted with a rubber cap and fixed by means of a cotton wool plug into the mouth of the disinfectant tube. These pipettes were specially made so that the external diameter at the mouth was constant in size ; it was then found that the size of the drop yielded was constant also, in the present instance = 0.02 c.c.

A disadvantage of this method, compared with the "garnet" method, is that the trace of disinfectant adhering to the sample is incubated along with it. In the "garnet" method these traces are removed by washing before incubation. However, in the case of phenol, this drawback was found to be unimportant, owing to the great dilution the sample underwent. At the beginning of the experiments the samples were one drop (= 0.02 c.c.) ; later, when the number of survivors was very much reduced, they were increased to 4 or 6 drops. Although in the case of anthrax spores the disinfectant, phenol, was very concentrated (5%), in the sub-culture plates the concentration was reduced to from 1 in 10,000 to 5 in 10,000, a concentration exerting no inhibition upon the development of anthrax spores¹.

TABLE I. (*Exp.* 15. 6. 07.)

Anthrax spores. 5% phenol. 20.2° C.

Sample No.	Amount of sample	Time elapsing hours	Nos. of bacteria counted (6 plates)	Mean	Mean no. of bacteria present in 1 drop disinfecting mixture	K^* , assuming reaction to be in accordance with the equation $-\frac{dn}{dt} = Kn$
						$\left(K = \frac{1}{t_2 - t_1} \log \frac{n_1}{n_2} \right)$
1	1 drop	0	441, 491, 489 365, 382	434	434, taken as initial value of n ($=n_1$) in calculating values of K	
2	1	0.5	429, 340, 361 462, 443, 426	410	410	0.049
3	1	1.5	276, 398, 360 351, 341, 379	351	351	0.061
4	1	2.7	310, 322, 338 351, 287, 378	331	331	0.044
5	1	3.92	324, 280, 294 277, 289, 329	299	299	0.041
6	2 drops	5.95	528, 501, 441 495, 451	483	241	0.043
7	4	25.6	61, 77, 149 127, 135, 125	112	28	0.046

Mean value of K , 0.047.

* In Tables I—XV, values of K from expression $\frac{1}{t_2 - t_1} \log \frac{n_1}{n_2}$ were calculated with Briggs' logarithms in place of natural logarithms.

In the case of anthrax it was found necessary to use as small an amount of agar as was convenient (viz. 8 c.c.), so that, after pouring, the film of agar in the petri dish formed as thin a layer as possible, and abundant oxygen was provided for the germination of the spores. The melted agar was poured at a temperature of about 65° C. ; this was done to obtain perfect mixing and separation of the spores in the drop sample. For the same reason, the plates were previously warmed to about 40° C., and 0.5 to 1 c.c. sterile distilled water placed near the middle of each ; the sample from the disinfectant tube was dropped into the water, and this instant was considered the exact moment of sampling. The sample was well stirred with a

¹ In the experiments with *B. paratyphosus* the concentration of disinfectant in the sub-cultures was quite insignificant, e.g., phenol 1 in 200,000 to 1 in 50,000, according to the size of the sample taken. When mercuric chloride was employed it had to be neutralised.

platinum needle, the agar then poured, and the whole well mixed. The correct enumeration of anthrax spores presents some trouble owing to difficulty in separation, and very many failures resulted before the above exact procedure was evolved and adhered to.

Six plates were poured of each sample, they were incubated for 24—48 hours at 37° C., and the mean of the six enumerations taken as the result.

The results of two experiments are given in Tables I and II, the values of "*K*" are calculated, as before, from the mean values of the numbers counted on the plates; the constancy of the value is exceedingly well marked.

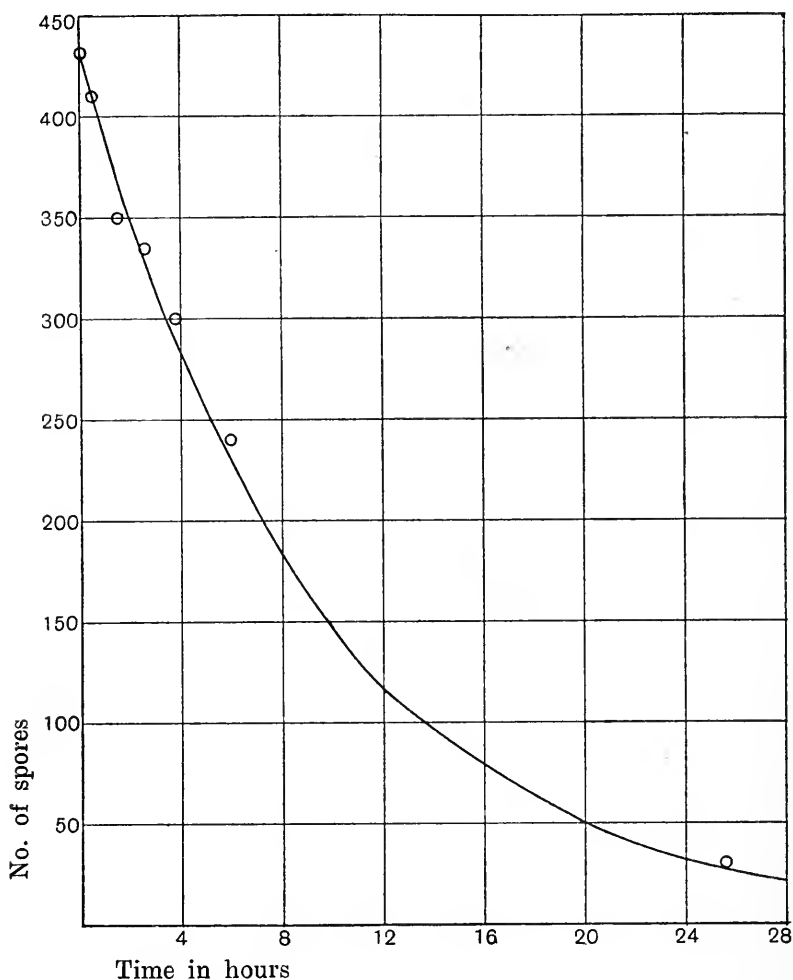


Fig. 2. (Exp. 15. 6. 07.) Showing course of disinfection of anthrax spores with 5% phenol at 20.2° C. (see Table I). The curve is drawn through a series of points found by calculation; the circles show the results of experimental determinations.

The close agreement of the results of these two experiments with the theory is also shown in Figures 2 and 3. In the experiment at 20.2° C. (Table I), the mean value of *K* was found to be 0.047. A curve

TABLE II. (Exp. 18. 6. 07.)
Anthrax spores. 5 % phenol. 33.3° C.

Sample No.	Amount of sample	Time elapsing hours	Nos. of bacteria counted, mean of 6 plates	Mean no. of bacteria present in 1 drop disinfecting mixture	K , assuming reaction to be in accordance with the equation $-\frac{dn}{dt} = Kn$ $\left(K = \frac{1}{t_2 - t_1} \log \frac{n_1}{n_2}\right)$
1	1 drop	0	439	439, taken as initial value of n ($=n_1$) in calculating values of K	
2	1	0.5	275.5	275.5	0.40
3	1	1.25	137.5	137.5	0.40
4	1	2	46	46	0.49
5	2 drops	3	31.6	15.8	0.48
6	2	4.1	10.9	5.45	0.46
7	4	5	13.9	3.6	0.41
8	6	7	3	0.5	0.42

Mean value of K , 0.44.

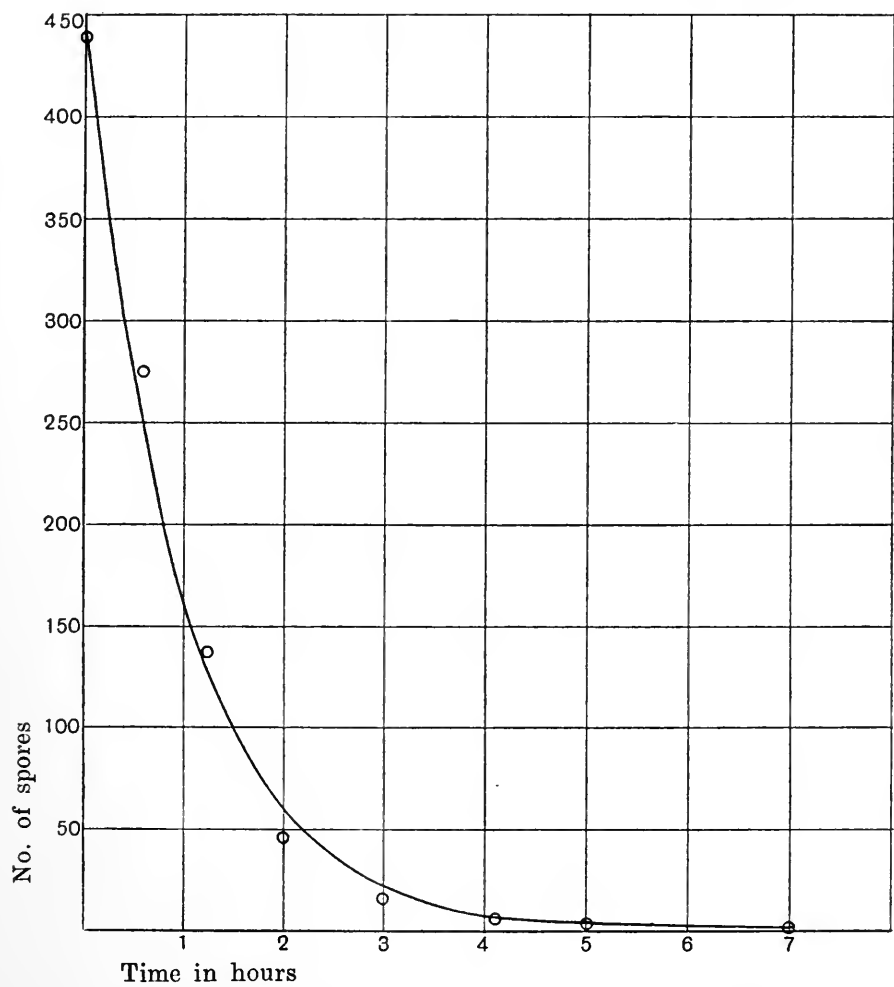


Fig. 3. (Exp. 18. 6. 07.) Showing course of disinfection of anthrax spores with 5 % phenol at 33.3° C. (see Table II). The curve is drawn through a series of calculated points, the circles show the results of experimental determinations.

(Fig. 2) was then drawn through a series of points obtained by putting $K = 0.047$ in the equation given on p. 95, thus:—

$$0.047 = \frac{1}{t_2 - t_1} \log \frac{n_1}{n_2},$$

n_1 , the initial number of organisms, being 434. The circles, Fig. 2, represent the points actually found by experiment. The curve in Fig. 3 was drawn in a similar way using the mean value of K obtained in the experiment at 33.3°C . There is an equally close agreement between the observed and calculated points in this instance also. One may therefore rely upon this method to give as consistent results as the “garnet” method.

In the case of anthrax spores the course of disinfection, either with mercuric chloride or phenol¹, proceeds in a perfectly orderly manner and can be represented by the equation:—

$$\frac{1}{t_2 - t_1} \log \frac{n_1}{n_2} = K.$$

Experiments with B. paratyphosus.

To determine whether the law thus obtained for disinfection of anthrax spores with two different disinfectants was capable of wider application, experiments were made with a non-sporing organism. *B. paratyphosus* was chosen for most experiments because its recognition is at all times facilitated by its property of fermenting dulcete and glucose.

The disinfectants chosen were: (1) phenol, (2) disinfectant “A” (an emulsified preparation of higher coal tar derivatives), (3) mercuric chloride.

A 24 hours’ broth culture was used, and in the earlier experiments this broth culture was used directly without dilution, and the samples from the disinfection tubes diluted before plating (Tables III, IV and XI).

¹ Phenol is a weak and uncertain germicide for spores and it was found necessary to use it as strong as possible, viz. 5%; even then the reaction at 20°C . was not complete after 28 hours. At 33°C . the disinfection proceeded much more rapidly and was nearing completion after 7—8 hours. These results roughly confirm those of previous workers using widely different methods: Koch (1886) showed phenol to be an untrustworthy disinfectant for anthrax spores, Fraenkel (1889) found them to be killed only in 40 days by 5% phenol, while Behring (1890) found that although at room temperature many days were necessary, at 37.5°C . disinfection was complete in 3 hours. These three workers all used the “thread” method. Krönig and Paul (1897), with the “garnet” method, showed the reaction with 5% phenol to be incomplete in 24 hours.

Dilution tubes, containing a suitable amount of sterile water, were prepared, and small amounts (1, 2 or 5 drops) added to them from the disinfection tubes by means of the capillary pipettes. From the dilution tubes, small quantities measured with capillary pipettes were plated. At the beginning of an experiment dilution was necessarily large, later on it became possible to plate samples from the disinfection tube undiluted. This method, which is very troublesome in practice and needs many trial experiments before a successful one is obtained, has the additional drawback that, whereas the object of the experiment is to obtain a series of perfectly comparable results, hardly any two of them are obtained by means of an entirely comparable procedure. There appears to be a very considerable error introduced with dilution and the use of capillary pipettes, probably due to a tendency for the suspended bacteria to be attracted to the walls of the pipettes, etc., consequently the drops yielded may not be true samples. However, if the same sampling pipette is always used, and no subsequent dilution takes place, the results are found to be fairly comparable. Later on therefore, the method was changed, the broth culture was extensively diluted before use, and a comparatively small number of organisms was placed in the disinfection tubes, less than a thousand per drop. The sample drops, all removed with the same pipette, were plated undiluted, and the plates, poured from time to time, being perfectly comparable, the results presented much greater consistency.

As has been said before (p. 97), in the case of phenol and disinfectant "A," it was not found necessary to neutralise the trace of disinfectant carried over with the sample into the test cultures, as separate experiments showed the proportion there present to exercise no inhibition upon growth. The sample drop or drops, from disinfection tube or dilution tube, were dropped upon the plate, and melted agar, about 12 c.c. at a temperature not exceeding 45° C., was immediately poured upon the plate, and the whole well mixed. As a rule duplicate plates were poured at the same instant, and this necessitated the help of one or more assistants.

In the case of mercuric chloride, the trace carried over in the sample required precipitation (see p. 123), for mercuric chloride in exceedingly small amount can effectually inhibit subsequent growth even when the organisms may have retained their vitality at the moment of sampling. Accordingly the samples were neutralised with ammonium sulphide, of which a sufficient amount was added to the dilution tube, when diluted samples were plated, or directly placed

upon the plate when drops from the disinfection tube were plated undiluted. In the latter case the sample drop or drops were dropped directly into the ammonium sulphide solution and melted agar immediately poured into the plate and the whole well mixed. The results of these enumeration experiments with all three disinfectants are given in Tables III—X; phenol, Tables III—VII; disinfectant "A," Tables VIII and IX; mercuric chloride, Table X.

The values of K given by the equation

$$K = \frac{1}{t_2 - t_1} \log \frac{n_1}{n_2}$$

are also given, and it will be seen that the value of K does not remain constant as was the case with anthrax spores, but continuously decreases as the experiment proceeds. This continuous fall in the value of K occurred in the case of all three types of disinfectants worked with.

TABLE III. (*Exp. 6. 2. 07.*)

B. parat. 24 hours' culture. Phenol, 6 per 1000. 20° C.

Time minutes	Mean no. of bacteria present in diluted samples plated		Mean no. of bacteria present in 1 drop original disinfection mixture	Values of K , assuming reaction to be in accordance with the equation		
				(a) $-\frac{dn}{dt} = Kn$	(b) $-\frac{dn}{dt} = Kn^{1.5}$	(c) $-\frac{dn}{dt} = Kn^2$
·5	121	× 125	15,000, taken as initial value of $n (=n_1)$ in calculating values of K			
1	110	× 50	5,500	—	—	—
2	238	× 25	5,950	—	—	—
3	41	× 25	1,025	0·27	0·009	0·00015
4	173	× 10	1,730	0·27	0·012	0·00024
5	86	× 10	860	0·26	0·012	0·00031
6	140	× 4	560	0·22	0·011	0·00038
7	131	× 4	524	0·22	0·013	0·00042
8	78	× 4	312	0·20	0·012	0·00024
9	76	× 4	304	0·18	0·011	0·00037
10	69	× 4	276	0·15	0·013	0·00056
15	25·5	× 4	102	0·12	0·011	0·00071
20	18	× 4	72	0·08	0·007	0·00048
30	—	—	71	0·06	0·007	0·00050

TABLE IV. (Exp. 1. 2. 07.)

B. parat. 24 hours' culture. Phenol, 6 per 1000. 20° C.

Time minutes	Mean no. of bacteria present in diluted samples		Mean no. of bacteria present in 1 drop disinfection mixture	K , assuming the reaction to be in accordance with the equation		
				$(a) - \frac{dn}{dt} = Kn$	$(b) - \frac{dn}{dt} = Kn^{1.5}$	$(c) - \frac{dn}{dt} = Kn^2$
1	54	× 50	2,700, taken as initial value of $n (=n_1)$ in calculating values of K			
2	12.5	× 50	625	0.63	0.042	0.0012
3	6	× 25	150	0.61	0.062	0.0031
4	5.5	× 25	137	0.42	0.044	0.0025
5	11.5	× 10	115	0.34	0.036	0.0021
7	5.5	× 10	55	0.25	0.038	0.017

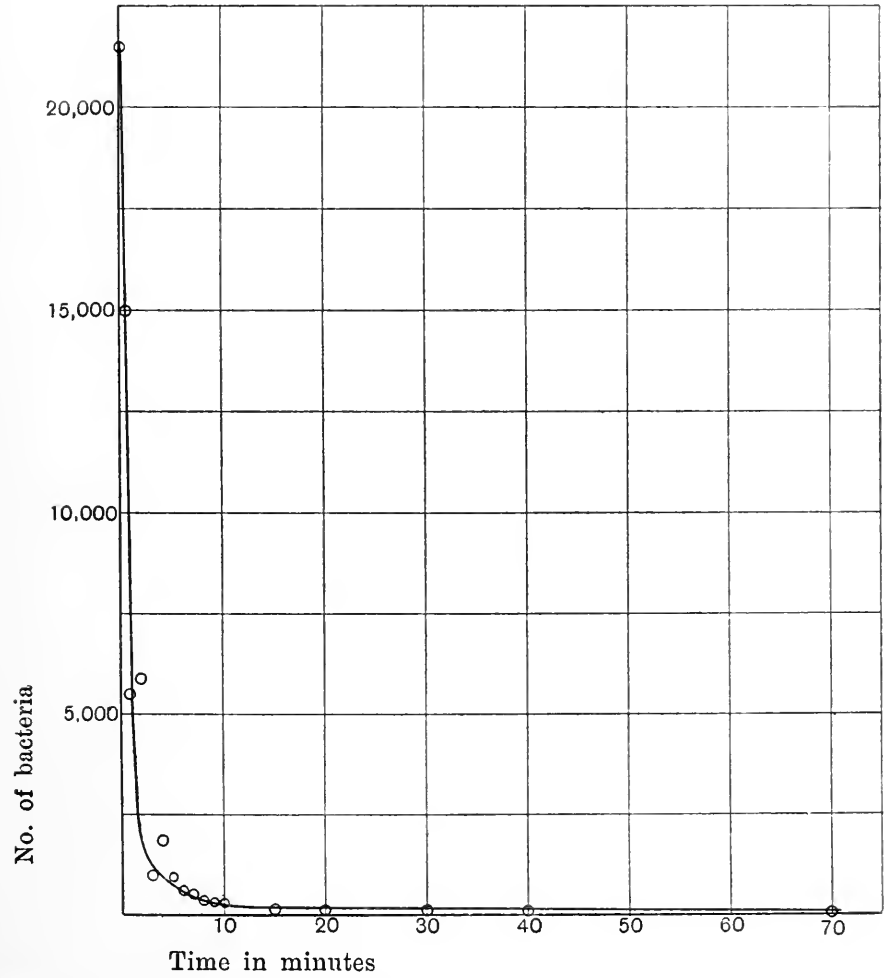


Fig. 4. (Exp. 6. 2. 07.) Disinfection of a 24 hours' culture of *B. paratyphosus* with phenol, 6 per 1000, at 20° C. (see Table III).

TABLE V. (Exp. 11. 7. 07.)

B. parat. 24 hours' culture. Phenol, 6 per 1000.

	Time mins.	Amount of sample taken	Nos. counted on plates	Mean	Mean no. of bacteria present in 1 drop disinfection mixture	K , assuming reaction to be in accordance with the equation $-\frac{dn}{dt} = Kn$
(a) 20° C.	1	1 drop	569, 509	539	539, taken as initial value of $n (=n_1)$ in calculating values of K	
	2	1	357, 247, 226	276.6	276.6	0.29
	3	1	151, 124	137.5	137.5	0.30
	4	2 drops	140, 167, 174	160.3	80.1	0.28
	6	3	139, 113	126	42	0.22
	8	5	83, 90, 93	88.8	17.8	0.21
	12	7	21, 14, 12	15.7	2.2	0.22
(b) 30° C.	1	1 drop	1252, 1480	1368	1368, taken as initial value of n	
	2	3 drops	473, 474, 509	485	162	0.93
	3	6	438, 352, 388	392.6	65.5	0.66
	4.1	6	117, 82, 73	90.6	15.1	0.63
	6	10	8, 13, 25	15.3	1.5	0.59

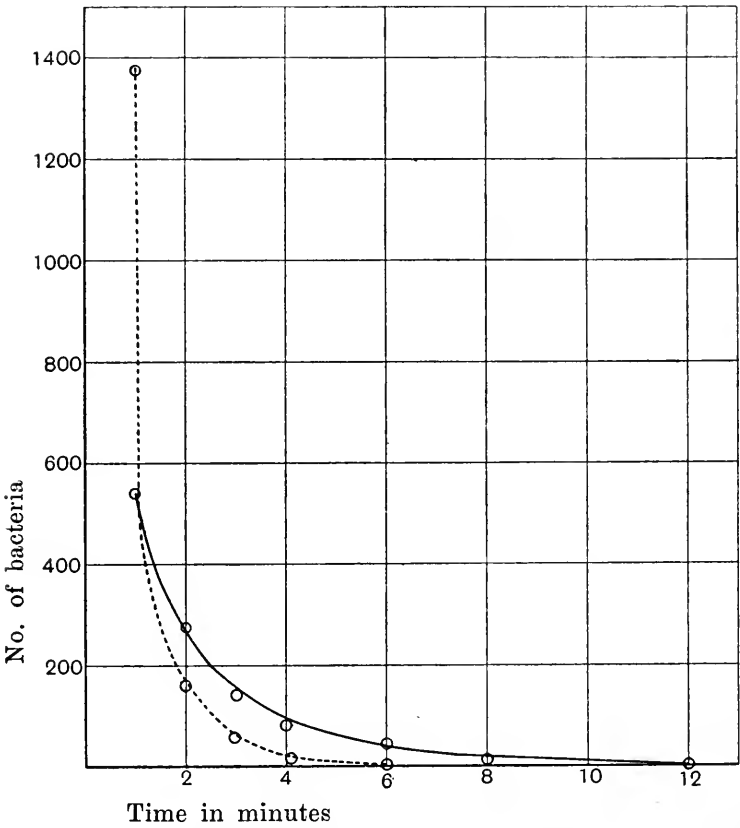


Fig. 5. (Exp. 11. 7. 07.) Disinfection of a 24 hours' culture of *B. paratyphosus* with phenol, 6 per 1000, at 20° C., continuous curve : at 30° C., dotted curve (see Table V).

TABLE VI. (*Exp.* 24. 7. 07.)

B. parat. 24 hours' culture. Phenol, 6 per 1000.

	Time minutes	Mean no. of bacteria present in 1 drop disinfection mixture	K , assuming reaction to be in accordance with the equation $-\frac{dn}{dt} = Kn$
(a) 11° C.	0	823, taken as initial value of n ($=n_1$) in calculating values of K	
	1.3	202	0.47
	2.3	80	0.44
	3.3	44.7	0.38
	5.3	14.3	0.33
	7.3	4.35	0.31
	10.3	1.0	0.28
	15.3	0.22	0.23
(b) 21° C.	0	974, taken as initial value of n	
	1.2	73	0.94
	2.2	16.3	0.81
	3.3	4	0.72
	4.3	0	—

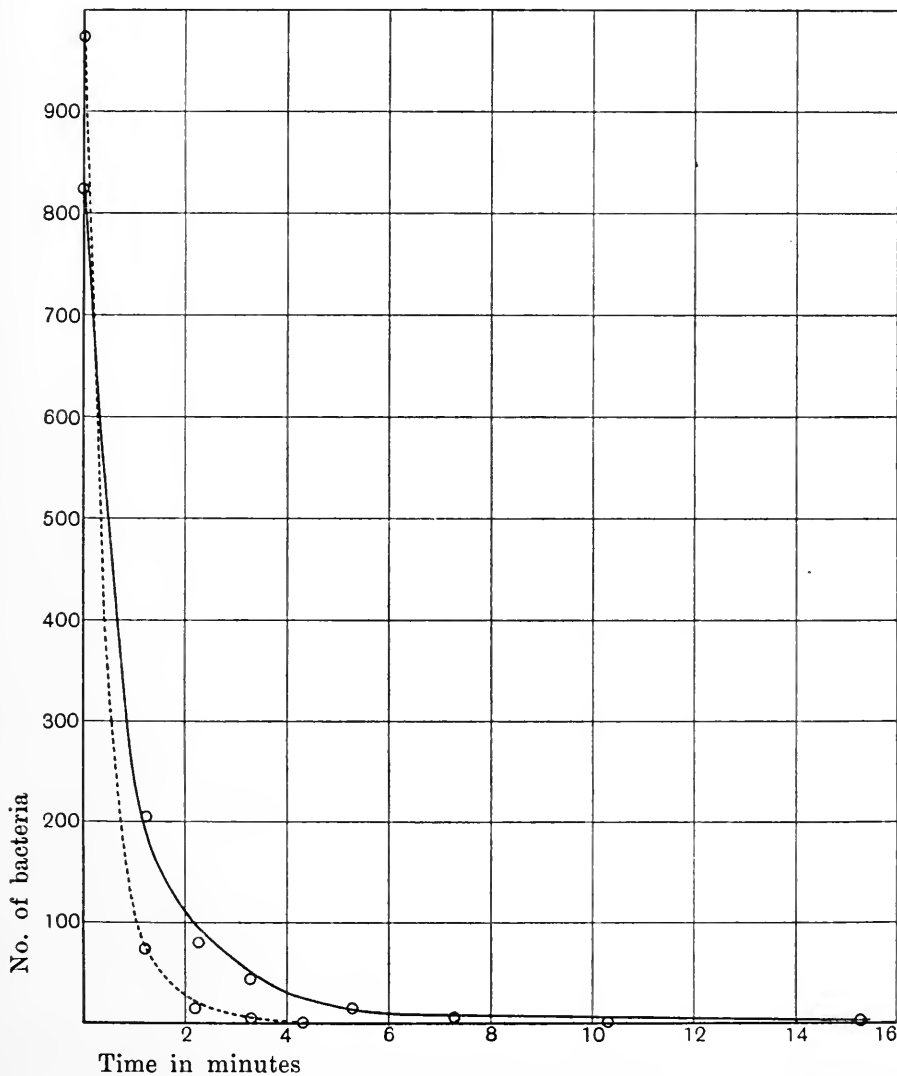


Fig. 6. (*Exp.* 24. 7. 07.) Disinfection of a 24 hours' culture of *B. paratyphosus* with phenol, 6 per 1000, at 11° C., continuous curve: at 21° C., dotted curve (see Table VI).

TABLE VII. (*Exp.* 23. 7. 07.)

B. parat. 24 hours' culture. Phenol, 6 per 1000.

	Time minutes	Mean no. of bacteria present in 1 drop disinfection mixture	K , assuming reac- accordance with t $-\frac{dn}{dt}=Kn$
(a)	11° C.		
	1	765, taken as initial value of n ($=n_1$ values of K	
	3	496	0.094
	5	271	0.112
	10	92.5	0.102
	20	5	0.062
	44	0.7	0.076
(b)	21° C.	484, taken as initial value of n	
	3	90	0.36
	5	28.5	0.31
	7	9.3	0.28
	10	3.5	0.24
	20.5	0.15	0.18

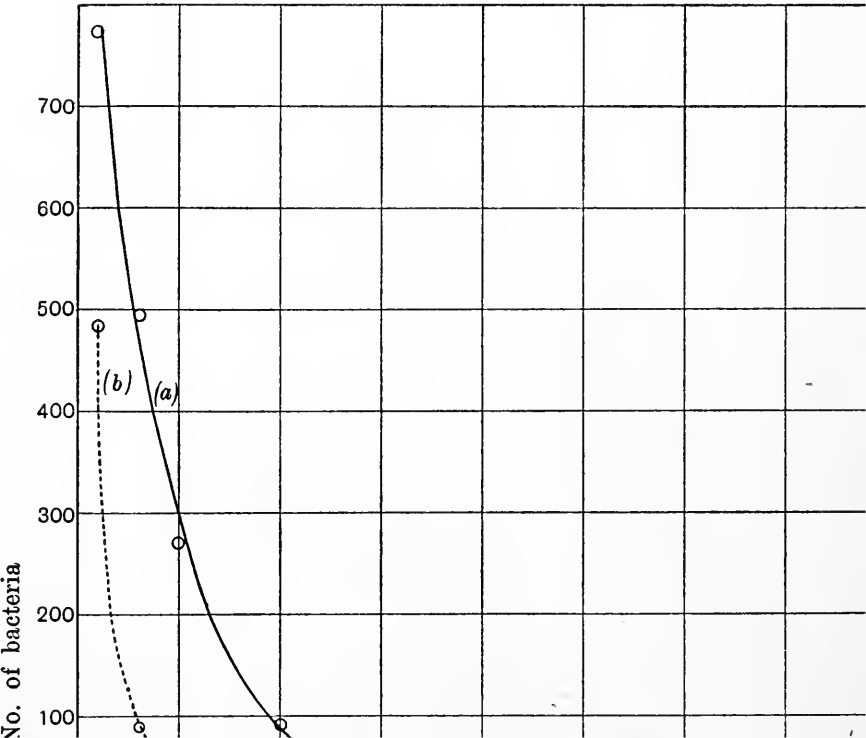
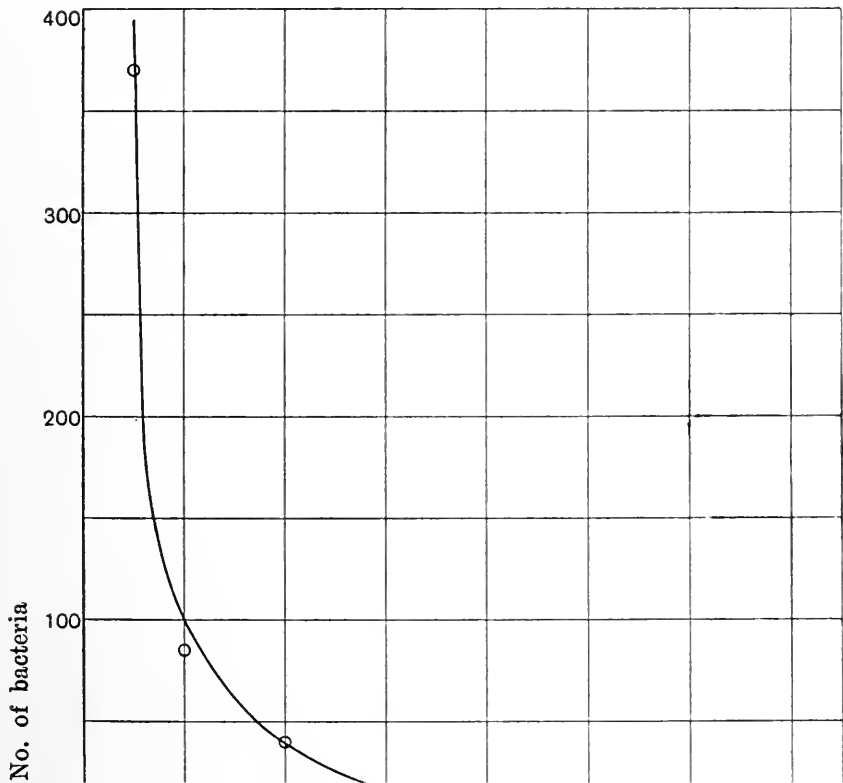


TABLE VIII. (*Exp.* 20. 3. 07.)*B. parat.* Disinfectant "A," 5.5 per 10,000. 20° C.

Time minutes	Mean no. of bacteria present in 1 drop disinfection mixture	K, assuming reaction to be in accordance with the equations			
		(a) $-\frac{dn}{dt} = Kn$	(b) $-\frac{dn}{dt} = Kn^{1.5}$	(c) $-\frac{dn}{dt} = Kn^{1.7}$	(d) $-\frac{dn}{dt} = K$
0.5	370, taken as initial value of $n (=n_1)$ in calculating values of K				
1	85	1.28	0.34	0.081	0.018
2	38.8	0.65	0.15	0.059	0.016
3	15	0.56	0.16	0.077	0.026
4	11	0.44	0.14	0.070	0.025
5.5	8	0.33	0.12	0.061	0.024
7	4	0.30	0.14	0.074	0.038



*Disinfection*TABLE IX. (*Exp.* 26. 3. 07.)*B. parat.* Disinfectant "A," 5·5 per 10,000. 20° C.

Time minutes	No. of bacteria present in 1 drop disinfection mixture	<i>K</i> , assuming reaction to be in accordance with the equation
		$-\frac{dn}{dt} = Kn$
0	25,250, taken as initial value of $n (=n_1)$ in calculating values of K	
0·5	540	3·74
1	305	1·92
2·1	97	1·15
3·1	50	0·87
4·1	24	0·74
5·2	10	0·65
7	2	0·57

TABLE X. (*Exp.* 23. 3. 07.)*B. parat.* Mercuric chloride, 2 per million. 20° C.

Time minutes	Mean no. of bacteria present in diluted sample		Mean no. of bacteria present in 1 drop disinfection mixture	K , assuming reaction to be in accordance with the equation $-\frac{dn}{dt} = Kn$
1·1	85	× 250	21,250, taken as initial value of n ($=n_1$) in calculating values of K	
2·1	82	× 250	20,500	*
3	68	× 250	17,000	*
4	52	× 250	13,000	0·074
5	53	× 250	13,250	0·050
7	62	× 250	15,500	0·023
10	97	× 125	12,125	0·027
15	96	× 125	12,000	0·017
20	69	× 100	6,900	0·026
30	139	× 50	6,950	0·018
42	305	× 25	7,625	0·011
51	279	× 25	6,975	0·010
60	276	× 10	2,760	0·015
80	215	× 10	2,150	0·012
93	130	× 10	1,300	0·013
140	—	—	150	0·015
180	—	—	49	0·015
210	—	—	29	0·013
245	—	—	29	0·012

* The values of n at these times are not sufficiently accurate to give consistent values for K .

The following hypotheses are advanced to account for the facts recorded above, viz. that in the case of anthrax spores, disinfection

proceeds in accordance with the equation $-\frac{dn}{dt} = Kn$, while in the case of *B. paratyphosus* this equation cannot be applied.

Let us assume that during disinfection the germicide operates upon the bacterium by so altering the constitution of its protoplasm (by chemical combination or otherwise), as to render it unfit for the continued vitality of the organism. The disinfectants, mercuric salts and phenols, used in this investigation form chemical compounds with proteins. It would not therefore be surprising that disinfection should conform to a law known to govern many chemical processes, and we may employ one of the well known interpretations as to why these reactions should be gradual and not sudden, viz., that at a particular time only a proportion of the molecules (of the bacteria in the case of disinfection) are temporarily in such a state as to permit of the combination¹. With *B. paratyphosus* we must presuppose the existence of another factor in addition. In this case it would appear that certain individuals are permanently more sensitive to the reaction than others, or, in other words, possess less resistance to the disinfecting process. The less resistant organisms would then be killed in greater proportion than the more resistant during the earlier stages of the reaction, and the value of K would progressively diminish. The nearer one could approach to uniformity of resistance, the more nearly constant would the value of K become (this being very nearly realised in the case of an emulsion of anthrax spores, especially after they have been subjected to a temperature of 80° C.).

In the case of a 24 hours' culture of *B. paratyphosus* permanently different resistances to disinfectants may conceivably be bound up with variation in age. In any collection of bacteria of differing resistance the velocity of disinfection will be proportional to the number of low resistant individuals present. This number, if age is the condition determining resistance, will again be a function of the total number present², and the course of the reaction will be expressed thus:—

$$-\frac{dn}{dt} = Kn \times f(n),$$

¹ This theory has received substantial support in the case of some radioactive substances, whose decay proceeds in accordance with the unimolecular law (see Rutherford, *Radioactivity*, pp. 182 and 338, 4th ed., 1904).

² The decrease in the value of K in the case of *B. paratyphosus* is a regular and orderly one. If values of K are plotted against numbers of surviving individuals, a continuous curve is obtained, showing that the value of K is altering in accordance with some law and bears some relation to the number of surviving bacteria.

instead of

$$-\frac{dn}{dt} = Kn \left(\text{or, on integration, } K = \frac{1}{t_2 - t_1} \log \frac{n_1}{n_2} \right),$$

which is the equation referring to the case where the individuals are of uniform resistance.

These hypotheses were confirmed by experiment.

If $f(n) = n^0$, the first equation becomes equal to the second one, and for this, as has been seen, the value of K in every case progressively decreased.

If $f(n) = n^1$, the equation becomes $-\frac{dn}{dt} = Kn^2$, and on integration we get $K = \frac{1}{t_2 - t_1} \left(\frac{1}{n_2} - \frac{1}{n_1} \right)$; applying this equation, the value of K was found in every case to progressively increase (see Tables III, VIII and XI).

Between the two values $f(n) = n^0$ and $f(n) = n$ a large series was tried, where $f(n)$ was placed equal to n raised to a series of powers between 0 and 1, *e.g.*, $n^{0.2}$, $n^{0.4}$, $n^{0.5}$, $n^{0.7}$, etc. In many cases the value of K was found to remain fairly constant when $f(n)$ was made equal to $n^{0.5}$ (*e.g.*, Tables III, IV and XI). The differential equation then has the form

$$-\frac{dn}{dt} = Kn^{1.5},$$

and on integrating

$$K = \frac{1}{0.5} \cdot \frac{1}{t_2 - t_1} \left(\frac{1}{(n_2)^{0.5}} - \frac{1}{(n_1)^{0.5}} \right).$$

In some cases (*e.g.*, Table VIII), a constant value for K was obtained when $f(n) = n^{0.7}$, *i.e.*

$$-\frac{dn}{dt} = Kn^{1.7},$$

and

$$K = \frac{1}{0.7} \cdot \frac{1}{t_2 - t_1} \left(\frac{1}{(n_2)^{0.7}} - \frac{1}{(n_1)^{0.7}} \right).$$

In some cases (Tables V and VII) the value of $f(n)$ lay between n^0 and $n^{0.5}$; putting $f(n) = n^{0.5}$ one obtained an ascending value for K .

The exact value of $f(n)$ was found to depend on details of the materials used, on the exact admixture of the different resistances in any collection of individuals. It depended upon the age of the culture and age of the seed material used for making the culture, and upon the exact period of the disinfection process which was under examination. The value, for example, may be different if K is calculated with a trustworthy control enumeration as n_1 , or if the initial number for calculation is the amount surviving after some time has elapsed.

This reasoning was further tested as follows: if the above hypotheses are true, then if by any means a number of *B. paratyphosus* organisms of exactly the same age could be obtained, the truth should again be expressed by the original formula,

$$-\frac{dn}{dt} = Kn,$$

or

$$K = \frac{1}{t_2 - t_1} \cdot \log \frac{n_1}{n_2}.$$

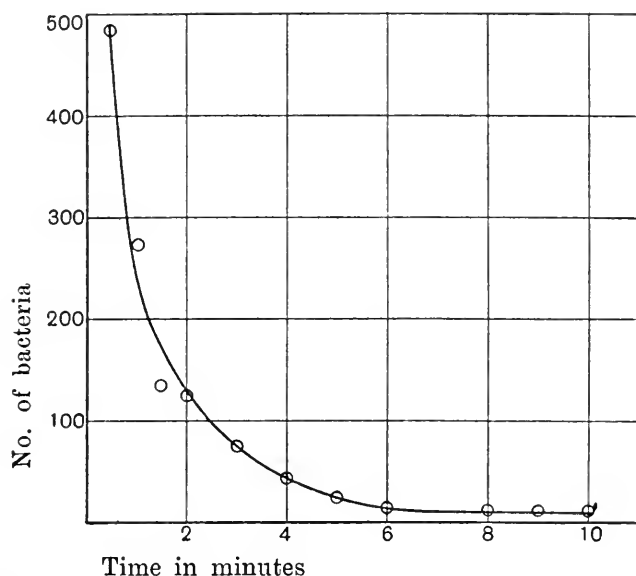


Fig. 9. (Exp. 6. 5. 07.) Disinfection of a 3 hours' culture of *B. paratyphosus* with phenol, 6 per 1000, at 20° C. (see Table XI).

TABLE XI. (Exp. 6. 5. 07.)

B. parat. 3 hours' culture. Phenol, 6 per 1000. 20° C.

Time minutes	Mean no. of bacteria present in 1 drop of disinfection mixture	Values of K , assuming reaction to be in accordance with the equations		
		(a) $-\frac{dn}{dt} = Kn$	(b) $-\frac{dn}{dt} = Kn^{1.5}$	(c) $-\frac{dn}{dt} = Kn^2$
0	1000, taken as the initial value of n ($=n_1$) in calculating values of K			
0.5	484	0.62	0.056	0.0021
1	272	0.56	0.058	0.0026
1.5	136	0.58	0.072	0.0042
2	128	0.46	0.056	0.0034
3	72	0.38	0.058	0.0043
4	46	0.33	0.058	0.0052
5	24	0.32	0.078	0.0081
6	16	0.30	0.072	0.0085
8	13	0.23	0.062	0.0095
9	10.5	0.22	0.062	0.0105
10	11	0.20	0.054	0.0090

This condition cannot be satisfied completely, but approximations can be attempted by using very young cultures. An experiment made with a 3 hours' culture (see Table XI) inoculated in the ordinary way,

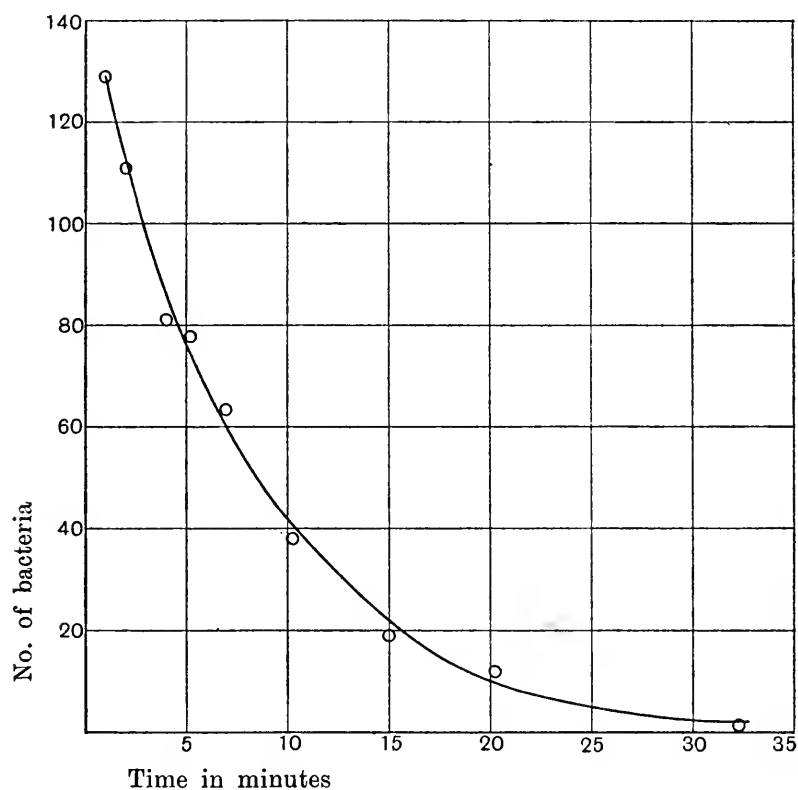


Fig. 10. (Exp. 30. 5. 07.) Disinfection of young individuals of *B. paratyphosus* with phenol, 6 per 1000, at 20° C. (see Table XII).

TABLE XII. (Exp. 30. 5. 07.)

B. parat. 3rd generation of young cultures. Phenol, 6 per 1000. 20° C.

Time mins.	Amount of sample taken	Numbers counted on plates	Mean	Mean no. of bacteria present in 1 drop disinfection mixture	K, assuming reaction to be in accordance with the equations	
					(a) $-\frac{dn}{dt} = Kn$	(b) $-\frac{dn}{dt} = Kn^{1.5}$
1	1 drop	147, 142, 99	129	129, taken as initial value of $n (=n_1)$ in calculating values of K		
2	1	114, 106, 112	111	111	0.066	0.014
3	1	94, 99, 97	97	97	0.062	0.013
4	1	100, 91, 57	83	83	0.064	0.015
5.1	2 drops	157, 182, 132	157	78	0.053	0.012
7	3	186, 215, 177	193	64	0.051	0.012
10.1	5	177, 190, 196	188	38	0.058	0.016
15	5	102, 141, 48	97	19	0.059	0.020
20.3	10	145, 154, 55	118	11.8	0.054	0.021
32.3	10	55, 42, 15	37	3.7	0.049	0.028

showed no essential difference from those made with 24 hours' cultures. In the next attempt the relatively large number of veterans added in the seed culture was avoided by passing the organism through several generations in broth, and sub-culturing with small amounts every three hours. It was thus possible to work with young cultures of the third and fourth generation.

The results of experiments with phenol are given in Tables XII—XVI, and it will be seen that the value of K given by the expression

$$K = \frac{1}{t_2 - t_1} \log \frac{n_1}{n_2},$$

although still decreasing in value during the experiments, does not decrease in value nearly so rapidly as was the case with 24 hours' cultures. The alternative equation

$$K = \frac{1}{0.5} \cdot \frac{1}{t_2 - t_1} \left(\frac{1}{(n_2)^{0.5}} - \frac{1}{(n_1)^{0.5}} \right), \text{ integral of } -\frac{dn}{dt} = Kn^{1.5},$$

applicable in many of the latter instances gave values of K which increased in value as the experiment proceeded (see Tables XII, XIII, XIV and XV *a*). The experiment in Table XII is given in detail, in XIII, XIV and XV *a*, results only are stated.

It is therefore clear that the age of the bacterium is a factor in determining its resistance to the action of a disinfectant. In the experiments (Tables XII—XV) made with young individuals, the value of the velocity constant is comparatively small, and approximates to that obtained towards the end of the reaction in more mixed cultures (*e.g.*, compare Table XII, initial value of $K = 0.066$, and falling to 0.049, with Table III, initial value of $K = 0.27$, falling to 0.06: concentration of phenol in both experiments the same, 6 per 1000: temperature in both instances, 20° C.).

This shows that it is the younger individuals which possess the higher resistance for disinfectants.

TABLE XIII. (*Exp. 7. 5. 07.*)

B. parat. 2nd generation of young cultures. Phenol, 6 per 1000. 20° C.

Time minutes	Mean no. of bacteria present in 1 drop of disinfecting liquid	Values of K , assuming reaction to be in accordance with the equations	
		(a) $-\frac{dn}{dt} = Kn$	(b) $-\frac{dn}{dt} = Kn^{1.5}$
1	1125, taken as initial value of $n (= n_1)$ in calculating values of K		
2	418	0.43	0.038
3	212	0.36	0.038
5	47	0.34	0.058
7	20	0.29	0.064

TABLE XIV. (*Exp.* 23. 5. 07.)*B. parat.* 3rd generation of young cultures. Phenol, 6 per 1000. 20° C.

Time minutes	Mean no. of bacteria in 1 drop of disinfection mixture	K, assuming reaction to be in accordance with the equations	
		(a) $-\frac{dn}{dt} = Kn$	(b) $-\frac{dn}{dt} = Kn^{1.5}$
1	166, taken as the initial value of $n (=n_1)$ in calculating values of K		
1.9	125	0.13	0.026
3.0	95	0.13	0.028
5.3	39	0.15	0.038
7.1	33	0.11	0.032
10	16	0.10	0.034
28	1.2	0.08	0.06

TABLE XV. (*Exp.* 27. 6. 07.)*B. parat.* 4th generation of young cultures. Phenol, 6 per 1000. 20° C.

Time minutes	Mean no. of bacteria present in 1 drop of disinfection mixture	K, assuming reaction to be in accordance with the equations	
		(a) $-\frac{dn}{dt} = Kn$	(b) $-\frac{dn}{dt} = Kn^{1.5}$
(a) 20° C. 1	135, taken as initial value of $n (=n_1)$ in calculating values of K		
3	93	0.081	0.018
5	61	0.086	0.020
7.5	33.6	0.093	0.026
10.2	28.3	0.072	0.022
12.3	25.2	0.065	0.020
15	16.6	0.065	0.022
20	10.3	0.059	0.024
25.3	11.6	0.044	0.017
30	8	0.042	0.022
45	0.5	0.055	0.060

TABLE XV. (*Continued.*)*B. parat.* 4th generation of young cultures. Phenol, 6 per 1000. 30° C.

	Time minutes	Mean no. of bacteria present in 1 drop of disinfection mixture	K , assuming reaction to be in accordance with the equation $-\frac{dn}{dt} = Kn$
(b)	30° C.	1	341, taken as the initial value of $n (=n_1)$ in calculating values of K
		2	166.5 0.31
		3	76 0.33
		5.4	8.4 0.38

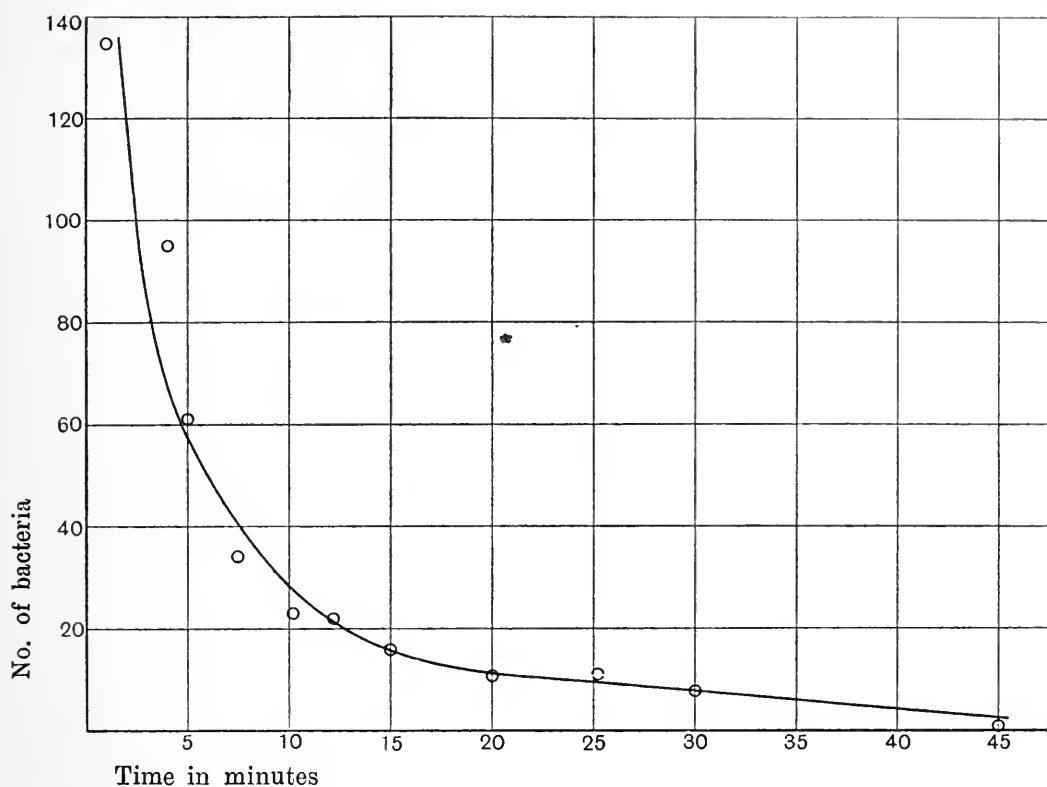


Fig. 11. (Exp. 27. 6. 07.) Disinfection of young individuals of *B. paratyphosus* with phenol, 6 per 1000, at 20° C. (see Table XV a).

An ideal case of disinfection, such as that of anthrax spores, may therefore be supposed, with experimental support, to proceed in accordance with the equation $-\frac{dn}{dt} = Kn$, an equation exactly similar in form to that expressing the course of a unimolecular reaction, or reaction of the first order. This latter equation is deduced directly from the Law of Guldberg and Waage: that the velocity of any reaction is at any moment proportional to the active mass of reacting substance present at that moment. For the case of disinfection one may then formulate a similar law: that the velocity of disinfection at any instant is proportional to the number (or weight) of living bacteria present.

An interesting parallel with the case of disinfection is found in such a reaction as that of the inversion of cane sugar with a dilute acid. This reaction is obviously dimolecular, so that the velocity, at any instant, should be proportional to the product of the active mass of the two reagents concerned, sugar and water. Inversion of cane sugar is, however, ordinarily so arranged that one reacting substance, the water, is present in so great an excess that one may consider its concentration

to remain constant during the course of the reaction, and the reaction velocity at any moment to be determined only by the active mass of the second reagent, the sugar, and hence to follow the law deduced for a unimolecular reaction. In a similar way in these experiments with disinfection the one reagent, the disinfectant, is present in an excess extremely great in comparison with the weight of bacterial substance present. The latter therefore governs the velocity of the process which in its nature shows an interesting analogy with that of a unimolecular reaction.

In the case of a non-sporing organism such as *B. paratyphosus*, experiments show that the reaction cannot be represented by this simple law, but that a permanent difference in resistance exists amongst individuals of different ages. The younger individuals possess greater resistance. When the conditions are so modified as to operate with individuals of approximately the same age the divergence largely disappears.

The gradual course of the disinfection process has been considered¹ to be completely explained by permanent variations of resistance in the bacteria employed, but it seems more probable that these differences in resistance are less significant than this, but sufficient to modify a process which would otherwise follow the above definite and constant logarithmic law.

Summary of Section I.

1. Disinfection is a process showing close analogy with a chemical reaction, the disinfectant representing one reagent, and the protoplasm of the bacterium the second.

2. It is a gradual process, without any sudden effects, and if the disinfectant is sufficiently dilute to admit of a reasonable time being taken for the process, the reaction velocity can be studied by enumerating the surviving bacteria at successive intervals of time.

3. In the case of disinfection of anthrax spores the reaction proceeds according to the well known equation for a unimolecular reaction embodying Guldberg and Waage's law. In this case "numbers of

¹ G. Bellei (1904), in the course of experiments on disinfection, repeatedly found that the greater the number of bacteria (*Staph. pyog. aureus*) disinfected, the longer was the time taken by the process. He explained the phenomena as due entirely to differences in resistance. One would rather explain it by considering it in the light of a reaction, where if more of the less abundant reagent be employed the time taken to complete the reaction will naturally be greater also.

surviving bacteria" are inserted in place of "concentration of reacting substance," i.e., the reaction velocity at any moment is proportional to the number of organisms surviving at that moment,

$$-\frac{dn}{dt} = Kn. \quad \text{Values of } K \left(= \frac{1}{t_2 - t_1} \log \frac{n_1}{n_2} \right),$$

calculated from the results of experiments, remained constant during the whole time of disinfection. This was the case with values calculated from experiments of

(a) Krönig and Paul, working with mercuric chloride, and using the "garnet" method.

(b) Madsen and Nyman, working with mercuric chloride and heat, and using Krönig and Paul's method.

(c) The present work, using phenol as disinfectant, a simple emulsion of spores, and the method described in the present work.

4. The process, although really involving two "reagents," follows the law of a unimolecular reaction, because the second reagent, the disinfectant, is present in so great an excess, comparatively, that its concentration may be regarded as unaltered during the process. An interesting analogy is thus offered with the case of the inversion of sugar, which, in reality a dimolecular process, obeys the laws relating to a unimolecular reaction for a similar reason.

5. Experiments with cultures of *B. paratyphosus* show a departure from this simple law, the reaction velocity diminishing during disinfection more rapidly than is accounted for by the fall in number of the surviving bacteria. This was the case with each of the three types of disinfectant used.

This divergence is due to differences in resistance between individuals of the various ages contained in such cultures.

SECTION II. THE EFFECT OF VARYING THE CONCENTRATION OF A DISINFECTANT UPON ITS GERMICIDAL ACTION.

Perhaps the best method to employ in investigating the effect of varying concentrations of disinfectant would be the comparison of the velocity coefficients of a series of different concentrations, using exactly similar groups of bacteria. This method is, however, almost impossible, owing to the labour and length of time that would be occupied by any one set of experiments. Another difficulty is that the reaction velocity, in the case of vegetative forms of bacteria, is changing in value through-

out the course of the disinfection, so that the velocity coefficients for a particular part only of the process, would be comparable. Accordingly, the method was adopted of measuring the time taken for almost complete disinfection of a constant number of bacteria, this time being considered to be inversely proportional to the mean reaction velocity of the process. This method justified itself by the concordant results which were obtained and has the practical advantage of yielding information specially with regard to the more resistant organisms of the culture. Exactly comparable experiments with a series of different strengths of disinfectant were made. The observations were made simultaneously and the same culture was used.

The method of experiment, except for a few details, was very similar to that of Rideal and Walker. One important difference was the introduction, as unit of measurement, of the drop from the standard pipettes (see p. 96) and the employment of much larger samples in the test cultures (4 drops = 0.08 c.c.). The experiments, moreover, extended over all lengths of time, whereas those of Rideal and Walker were confined to concentrations giving disinfection within fifteen minutes.

The same three types of disinfectants were again investigated and the organism generally used was *B. paratyphosus*, though a few results were also obtained with *Staphylococcus pyogenes aureus*.

A culture of standard resistance was obtained as follows: stock cultures upon sloped agar were maintained at room temperature, and from these a standard loopful was inoculated into 6 c.c. of standard broth and incubated exactly 24 hours at 37° C. This culture was immediately cooled to about 10° C. and then used for experiment.

5 c.c. of disinfectant solution was always employed and to this was added from a standardised pipette 5 drops (0.085 c.c.) of the broth culture, or about 30,000,000 organisms. A series of tubes were prepared containing different concentrations of disinfectant. These were inoculated as nearly as possible at the same time and maintained in a thermostat at constant temperature, viz., 20° C.

At suitable intervals of time samples, 4 drops from the standard pipettes (0.08 c.c.), were withdrawn, added to tubes of dulcete or glucose broth and at once incubated at 37° C. If the sample had contained any living organisms, growth (as evidenced by acid and gas production in either medium in the case of *B. paratyphosus*, and acid production in glucose broth in case of *Staph. pyogenes aureus*) was usually apparent in 24 hours; the tubes were, however, always kept for four days.

The sample taken (0.08 c.c.) represented about 1/60 of the total liquid present in the disinfection tube; a negative result in the test culture indicated therefore that about 30,000,000 bacteria had been reduced to less than 60 individuals. The times measured refer therefore to this practical end-point, which represents almost complete disinfection.

TABLE XVI.

Phenol. *B. paratyphosus*. 20° C. 5 drops (.085 c.c.) of a 24 hours' broth culture (about 30,000,000 bacteria) added to 5 c.c. disinfectant solution.

	Parts phenol per 1000 = C	Time taken for disinfection, <i>i.e.</i> reduction in number of about 30,000,000 individuals to less than 60 = t	Values of expression $\frac{1}{C_0 - C_n} \log \frac{C_n t_n^*}{C_0 t_0}$, where initial concentration of phenol, $C_0 = 12$, initial time, $t_0 = 3.25$
Exp. 21. 6. 06	12	3.25 minutes	—
	11	5.5	0.19
	10	8.5	0.17
	9	20.5	0.22
	8	68	0.28
	7	126	0.27
	6	240	0.26
	4.5	? 19 hours	—
	4	? 19	—
	3	75	0.28
Exp. 6. 6. 06	8	45 minutes	$C_0 = 8$; $t_0 = 45$
	7.5	75	0.39
	7	105	0.31
	6.5	125	0.24
	6	225	0.29
	5.5	440	0.29
	5	11.5 hours	0.33

* These and all subsequent values of the expression are calculated with Briggs' logarithms.

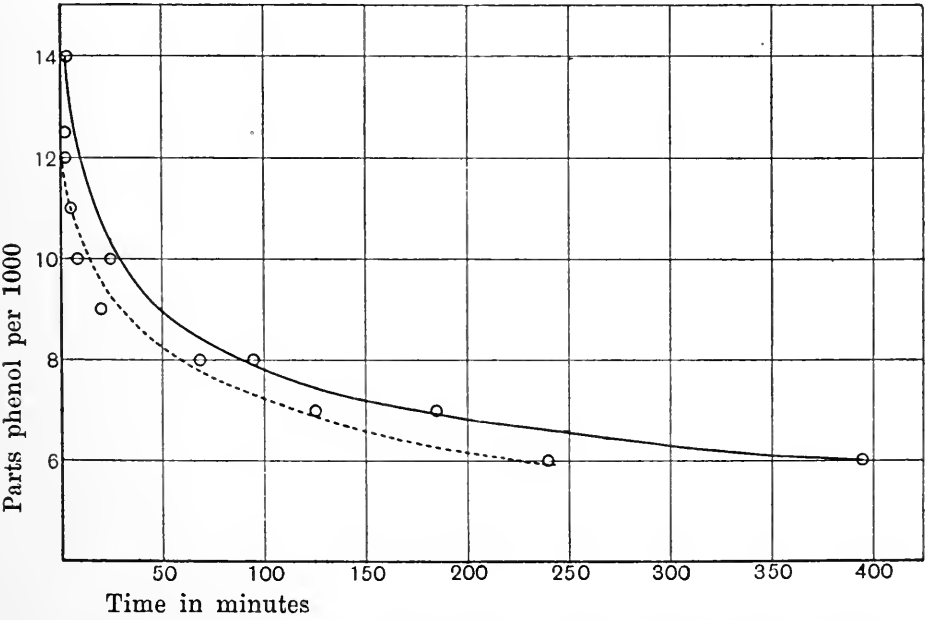


Fig. 12. Effect of varying the concentration of phenol upon the time taken for disinfection of (a) *B. paratyphosus*, dotted curve (Exp. 21. 6. 06, Table XVI): (b) *Staph. aureus*, continuous curve (Table XVII).

(1) *Phenol*.

The results of experiments with phenol are given in Table XVI (*B. paratyphosus*) and Table XVII (*Staph. pyogenes aureus*) and shown graphically in Fig. 12. The disinfection times given in the tables are the means between the time of last positive and first negative sub-culture. In some cases, where skipping occurred, each end-point was separately determined and the result given is the mean of the separate determinations. In these and similar tables where more than one experiment is given they were always made at different times with other but quite similar cultures.

TABLE XVII.

Phenol. *Staphylococcus pyogenes aureus*. 20° C.

Phenol parts per 1000 = C	Time taken for disinfection = t	Values of expression
		$\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0}$, where initial value of C , $C_0 = 14$, initial value of t , $t_0 = 4.5$
14	4.5 minutes	—
12.5	2.5	—
10	25	0.15
8	95	0.16
7	186	0.20
6	395	0.20
4	23.7 hours	0.19

It is evident that within the range observed small variations in concentration of disinfectant caused large differences in the time of disinfection. The relation between these concentrations and the corresponding times when plotted (Fig. 12, ordinates = concentrations of disinfectant: abscissae = times of disinfection) make continuous curves of similar form in the case of either organism. The curves suggested a logarithmic relation and the following equation, arrived at with the kind assistance of Dr J. C. G. Ledingham, was found to apply with very fair accuracy:

$$(\text{a constant})^C = Ct,$$

$$\text{or} \quad \frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0} = \text{a constant},$$

where t_0 and t_n are times for disinfection corresponding to concentrations C_0 and C_n . This equation was obtained in an empirical manner; its marked symmetry, however, suggests that it also has some physical significance.

The values of this expression calculated from the experiments were approximately constant (see Tables XVI and XVII). Considering the difficulty in accurately determining end-points in a process so gradual towards the end as disinfection was shown to be in Section I, the agreement with the above formula is maintained too well to be explained by mere coincidence.

TABLE XVIII.

Disinfectant "A." *B. paratyphosus*. 20° C.

	Parts "A" per 10,000 = C	Time taken for disinfection = t	Values of expression
			$\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0}$, where initial value of C , $C_0 = 10$ and initial value of t , $t_0 = 1.5$
Exp. 13. 9. 06	10	1.5 minutes	—
	8	2.5	—
	7	9	0.21
	6	12.5	0.17
	5	162	—
	2.5	more than 4 days	—
	1	" " "	—
Exp. 18. 9. 06	5	44 minutes	—
	5	simultaneous 210	—
	5	experiments 75	—
	5	105	—
Exp. 19. 9. 06	5.5	110 minutes	—
	5	205	—
	4.5	365	—
	4	500	—
	3	more than 5 days	—

TABLE XIX.

Disinfectant "A," another sample. *B. paratyphosus*. 20° C.

Parts "A" per 10,000 = C	Time taken for disinfection = t	Values of expression
		$\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0}$, where initial value of C , $C_0 = 10$, initial value of t , $t_0 = 1.25$
10	1.25 minutes	—
8	1.75	—
7	5	0.15
6	30	0.26
5	84	—

(2) *Disinfectant "A."*

In the case of disinfectant "A" (see Tables XVIII and XIX) there was only a small range of concentrations over which the corresponding

times of disinfection could be conveniently measured, *e.g.*, a concentration of 10 in 10,000 disinfected in 1.5 minutes, while 2.5 in 10,000 disinfected in 4 days.

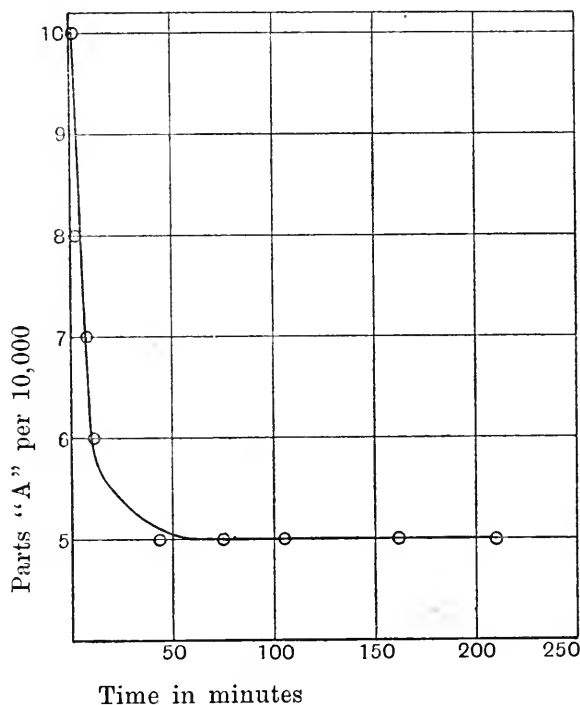


Fig. 13. (Exp. 13. 9. 06 and 18. 9. 06.) Effect of varying concentration of disinfectant "A" upon the time taken for disinfection of *B. paratyphosus* (Table XVIII).

The relation between concentration and time taken is different from that obtaining in the case of phenol. With disinfectant "A" (see Fig. 13) dilution has comparatively little effect upon the course of disinfection up to a certain point (*e.g.*, "A" 10 per 10,000 to "A" 5 per 10,000), then suddenly the curve becomes horizontal and the end-points of the reaction are in consequence difficult to determine accurately (see Table XVIII, Exp. 18. 9. 06). Two different samples of "A" were examined and both showed the same properties.

The number of determinations that can be made with any accuracy are necessarily limited, but if the portion of the curve between the two asymptotes be examined the values of the expression

$$\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0}$$

remains fairly constant in value (Table XVIII, Exp. 13. 9. 06, and Table XIX).

(3) *Metallic Salts* (mercuric chloride HgCl_2 , and silver nitrate AgNO_3).

Salts of the heavy metals, even when present in a nutrient medium in extremely small amount, exert an inhibitory influence upon the growth of bacteria. This fact was observed by Koch (1881), Behring (1890), Geppert (1889 and 1891) and others. The extent of this inhibition may be gathered from the fact that in my own experiments with mercuric chloride a concentration of 1 in 1,000,000 in the culture medium was found to prevent growth when about 20 individuals (*B. paratyphosus*) were added; a concentration of silver nitrate of 2 in 1,000,000 prevented growth when about 40 individuals were added.

In the case of phenol and the disinfectant "A" this phenomenon of inhibition by low concentrations of the disinfectant was found to be negligible. Control experiments, which were undertaken to test this point, showed that in the case of the most concentrated solutions employed, no error was introduced by the carrying-over of the disinfectant in making the test culture.

In the case of mercuric chloride, or silver nitrate, however, it is essential that the metal be thrown out of solution at the moment of sampling, and, with all but the most dilute solutions, it was necessary to add a large excess of precipitant over and above the amount actually required for the reaction. This fact, noticed by Geppert (1889 and 1891) is well shown in Table XX, where the time taken for disinfection by a definite concentration of mercuric chloride is seen to vary in proportion to the excess of precipitant employed.

TABLE XX.

Effect of adding varying excess of ammonium sulphide to neutralise mercuric chloride on sampling.

Concentration of mercuric chloride in disinfection tube	Amount of AmHS necessary to neutralise HgCl_2 carried over	Excess AmHS added over and above * necessary amount	Apparent time taken for disinfection
1 in 1000	0.4 drop	1.00 drop	less than 7 minutes
1 in 1000	0.4	2.00 drops	25 minutes
1 in 10,000	0.04	0.96 drop	less than 5 minutes
1 in 10,000	0.04	1.96	29 minutes
1 in 10,000	0.04	2.96 drops	56 „
1 in 10,000	0.04	3.96	56 „

* An even number of drops was actually added in each case, the AmHS solution being suitably diluted when necessary.

In addition to the inhibitory action upon the growth of bacteria exercised by traces of metallic salts, there is another phenomenon

exhibited by this class of disinfectant. If bacteria are subjected to the action of 1 in 1000, 1 in 10,000, or even weaker solutions of mercuric chloride, there is an interval during which some at least of them may be resuscitated by the timely administration of an antidote (in this case a sulphide solution), but if this antidotal treatment is not employed, no amount of dilution beyond the limits where inhibition occurs can prevent the death of the organism. It would seem that the mercuric salt has been already absorbed by the bacterium and possibly formed some combination with its substance, not, however, to a sufficient extent to prevent recovery if a large excess of the sulphide solution be employed.

The following two experiments illustrate this phenomenon :

Experiment I. 20. 3. 07. (a) A 24 hours' broth culture of *B. paratyphosus* was so diluted that in one standard drop there were 20 organisms. One such drop was added to tubes containing 10 c.c. glucose broth together with varying small amounts of mercuric chloride; inhibition took place when concentration of mercuric chloride in the culture tube reached 1 in 1,000,000.

(b) 5 c.c. of 1 in 1000 mercuric chloride solution was placed in the thermostat at 20° C. and inoculated with about 16,000,000 organisms (5 drops of broth culture). The complete disinfection tested by means of sub-cultures precipitated with H₂S, was found to take at least 5 minutes. Between 0.5 and 1.25 minutes after the start, samples of 4 drops (0.8 c.c.) were added to

(1) 900 c.c. glucose broth,

(2) 900 c.c. glucose broth, containing 0.15 c.c. H₂S solution.

In (2), 24 hours' incubation showed abundant formation of acid and gas; in (1) no growth was apparent after many days incubation.

Experiment II. (a) A drop from a diluted culture, containing 25 bacteria (*B. paratyphosus*), was added to 900 c.c. glucose broth to which 4 drops (0.8 c.c.) HgCl₂ 1 in 1000 had previously been added. The result on 24 hours' incubation was abundant acid and gas formation. A similar experiment with 50 organisms gave the same result.

(b) About 16,000,000 bacteria of the same culture, *B. paratyphosus*, were added to 5 c.c. of 1 in 1000 mercuric chloride at 20° C. The disinfection was found to take 7.5 minutes. About one minute after the start 4 drops (0.8 c.c.) were added from the disinfection tube to

(1) 900 c.c. glucose broth,

(2) 900 c.c. glucose broth, containing 0.15 c.c. H₂S solution.

The result was just the same as that of Exp. I: (1) remained sterile, while in (2) acid and gas were apparent after 24 hours' incubation.

In these two experiments, the dilution of 0.08 c.c. mercuric chloride (1 in 1000) by 900 c.c. of broth produced a concentration of mercuric chloride in the broth of 1 in 11,000,000, a concentration which did not produce inhibition (Exps. I (a) and II (a)) even when very few bacteria

were present. After a sojourn of one minute in 1 in 1000 mercuric chloride, exactly similar conditions of sub-culture failed to reveal the existence of any living bacteria (Exps. I (b 1) and II (b 1)), although the sub-cultures received a comparatively large number of organisms, which could have recovered their vitality if sulphide had been administered as an antidote (Exps. I (b 2), II (b 2)).

A difficulty in using large excess of these sulphide precipitants is that they themselves are, to a certain degree, inhibitive to bacterial growth. The following plan was therefore adopted in every case, whatever the concentration of mercuric chloride. A constant amount of precipitant was used, viz., the amount shown by separate experiments (with very few, about twenty, organisms) to be the maximum that could be safely employed without fear of inhibition.

The two sulphides generally used were yellow ammonium sulphide and hydrogen sulphide water. Solutions of these sulphides are unstable, hence they were always titrated before use (AmHS with HgCl_2 solution, lead acetate paper being used as an indicator; H_2S water directly with standard iodine solution). Sodium and potassium sulphides were also tried, but proved unsuitable owing to the solubility of mercury sulphide in the excess necessarily employed. According to the precipitant used, whether yellow ammonium sulphide or hydrogen sulphide, the disinfecting power of mercuric chloride was found to be very different in amount, and it appeared a question whether the power of the precipitant to decompose the bacteria-mercury compound was being determined rather than the disinfecting power of the original mercuric chloride.

Tables XXI and XXII show the effect of varying the concentration of mercuric chloride upon the time taken for disinfection, when the precipitants used are yellow ammonium sulphide and hydrogen sulphide respectively, and it will be seen that the germicidal value of mercuric chloride is different in the two instances. Yellow ammonium sulphide (Table XXI), *in a certain condition*, would appear to be a more effectual antidote for sublimated bacteria than hydrogen sulphide (Table XXII). It is, however, very difficult to obtain ammonium sulphide in precisely the right condition and it is very unstable. Possibly a certain amount of polysulphide should be present so that the small amount of dissolved mercury sulphide may contain no Hg^{++} ions. On account of this uncertainty ammonium sulphide was abandoned and hydrogen sulphide adopted as a standard precipitant. A saturated solution in distilled water ($= \frac{1}{2}$ normal) was easily prepared and kept in small stoppered bottles in the dark. 0.15 c.c. of this solution to 10 c.c. glucose broth

was found to be a sufficient excess to precipitate any of the strengths of sublimate worked with and to exercise no inhibition (tested with 10—20 organisms) on its own account.

TABLE XXI.

Mercuric chloride. *B. paratyphosus*. 20° C. Precipitant, yellow ammonium sulphide.

	Parts HgCl ₂ per 1000	Time taken for disinfection
Exp. 12. 12. 06	50	4.5 minutes
	10	17
	1	27
	0.1	> 127
Exp. 14. 12. 06	10	17
	1	56

TABLE XXII.

Mercuric chloride. *B. paratyphosus*. 20° C. Precipitant, H₂S (0.15 c.c. saturated solution added to each sub-culture tube containing 10 c.c. glucose broth).

	Parts HgCl ₂ per 1000	Time taken for disinfection = <i>t</i>	Nos. expressing concentration of Hg ⁺⁺ ions (Luther & Kahlenberg)	$\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0}$, where initial concentration of Hg ⁺⁺ ions, $C_0 = 63$, initial time, $t_0 = 1.5$
Exp. 27. 2. 07	1	1.5 mins.	63	—
	0.5	7	57.5	*
	0.1	13	42.5	0.037
	0.05	10	†37	0.023
	0.01	65	†23	0.030
	0.005	230	†16.5	0.034
Exp. 2. 3. 07	1	2.5	63	$C_0 = 63$, initial time, $t_0 = 2.5$
	0.5	7	57.5	*
	0.1	9.5	42.5	0.020
	0.05	22	†37	0.027
	0.03	57	†33	0.036
	0.01	> 150	—	—
	0.005	347	†16.5	0.034
	0.001	> 450	—	—

* Numbers obtained were not concordant, probably due to errors in measuring short times of disinfection.

† Numbers obtained by extrapolation.

The examination of the results obtained (see Tables XXI and XXII) shows that when the method of the present research is employed, lower values are obtained for the germicidal value of HgCl₂ than those of Koch (1881), Behring (1890), and Geppert (1889). From Table

XXI the astonishing fact is discovered that in a 24 hours' culture of *B. paratyphosus* some individuals at least are able to manifest vitality after contact with 5% mercuric chloride for 4 minutes, when ammonium sulphide is immediately applied as an antidote. When hydrogen sulphide is used, mercuric chloride is also seen (Table XXII) to have a considerably lower germicidal value than has usually been attributed

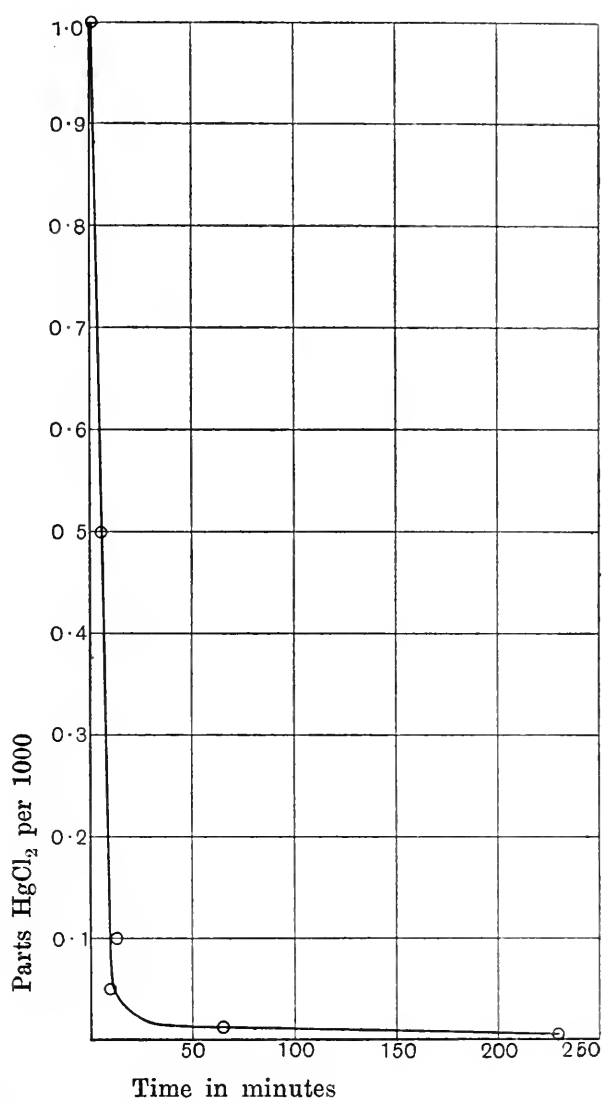


Fig. 14. (Exp. 27. 2. 07.) Times taken for disinfection of *B. paratyphosus* with varying concentrations of mercuric chloride, H₂S being used as precipitant (Table XXII).

to it. The difference in the results shown in Tables XXII and XXI is due to the different antidotal powers of hydrogen sulphide and the particular sample of ammonium sulphide employed. This again is doubtless due to different efficiency of the two reagents in decomposing a fairly stable compound formed by mercuric chloride and some con-

stituent of the bacterium. The value of corrosive sublimate as a practical disinfectant, however, remains unassailed.

The effect of altered concentration upon the time taken for disinfection when hydrogen sulphide is the precipitant is graphically shown in Fig. 14. The curve is very different in form to that obtained for phenol, and the formula $\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0} = \text{constant}$, was found to be quite inapplicable. Confirming an idea originally put forward by Dreser (1893), Krönig and Paul (1897) and Paul and Prall (1907) have published experiments showing that in the case of mercury salts, the mercuric ion¹, rather than the salt, is the real disinfecting agent. These workers employed *Staphylococcus pyogenes aureus* and anthrax spores and showed: (a) Mercuric salts, when arranged in order either of their electrolytic dissociation or of their disinfecting value, form two similar series. (b) Any procedure which diminishes the dissociation of mercuric chloride, such as the addition of sodium chloride or the substitution of alcohol for water as solvent, also diminishes the disinfecting power.

It therefore seemed probable that the difference in the relation between concentration and reaction velocity in the case of phenol and mercuric chloride might disappear if the mercuric ions were regarded as the real disinfecting agent. I am indebted to Dr N. T. M. Wilsmore of University College for figures representing the concentration of Hg^{++} ions corresponding to various concentrations of mercuric chloride, obtained from the results of Luther (1904) and Kahlenberg (1901). As the determinations of the latter worker were made at 95° C. they were reduced to the required temperature. The combined results of both workers were then plotted and from the curve values were obtained corresponding to the concentrations of mercuric chloride here employed; unfortunately only extrapolation values were available for the lower concentrations. In Table XXII values of the expression $\frac{1}{t_n - t_0} \log \frac{C_n t_n}{C_0 t_0}$ are given, where numbers representing concentrations of mercuric ions are substituted for concentrations of mercuric chloride, and it will be seen that a fair constancy is maintained, showing that disinfection by mercuric chloride is not very unlike that by phenol when the real agent is taken into account.

¹ That H^+ ions are the real toxic agents in disinfection by the mineral acids appears probable from the experiments of Bial (1897 and 1902) and Winslow and Lockridge (1906).

Silver nitrate. The validity of the arguments given above can also be tested by investigating the relation between concentration and rate of disinfection in the case of such a metallic salt as silver nitrate. Here the electrolytic dissociation in dilute solution is so complete that the concentration of metallic ions will be in proportion to the concentration of the salt.

In working with silver nitrate the procedure had to be modified owing to precipitation of the silver by the trace of broth introduced with the bacteria. An emulsion of bacteria in distilled water was therefore substituted for the drops of broth culture and it was obtained as follows: from the stock culture a broth culture was inoculated with a standard loopful and incubated for 24 hours at 37° C. ; from the broth culture a standard loopful was inoculated upon sloped agar tubes and also incubated for 24 hours at 37° C. This agar culture was emulsified with 2 c.c. of distilled water, filtered through muslin and centrifugalised for about 45 minutes. The water was removed, the deposit again emulsified with 2 c.c. distilled water, again filtered and shaken with glass beads. This emulsion when diluted fifteen times with distilled water was found to contain a concentration of bacteria about equal to that of the standard 24 hours' broth culture. Five standard drops were used for each experiment and found to contain 20—40 million bacteria.

The silver salt carried over in the test cultures was found to be most conveniently precipitated as sulphide ; 0.15 saturated solution of H_2S in water was added to each tube of glucose broth containing 10 c.c.

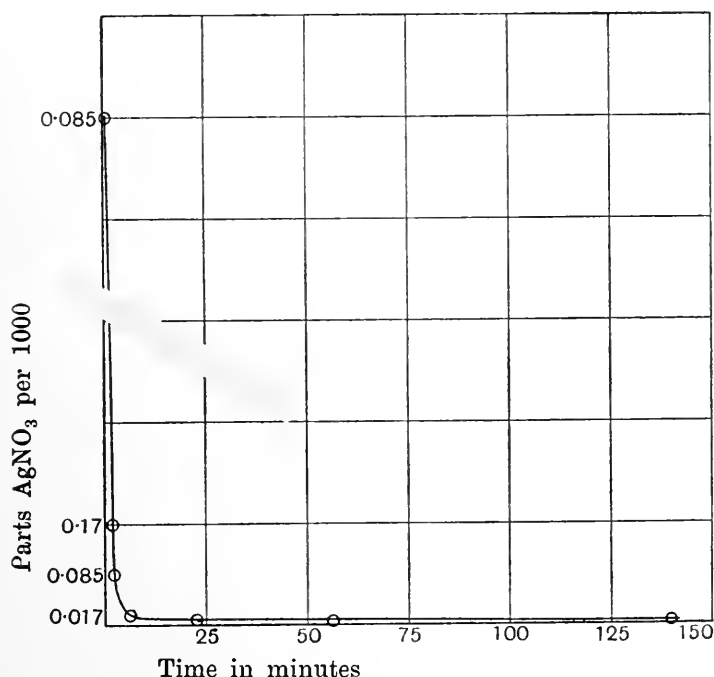


Fig. 15. (Exp. 24. 4. 07.) Times taken for disinfection of *B. paratyphosus* with varying concentrations of $AgNO_3$, H_2S being used as precipitant (Table XXIII).

The results are given in Table XXIII, where also are shown the values of the expression $\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0}$, and these show a very constant value. This constancy, in a case where the concentration of Ag^+ ions may be taken as approximately proportional to the concentration of the silver salt, is an interesting confirmation of the theory substantiated in the case of mercuric chloride, viz., that in cases of disinfection by metallic salts it is the metallic ion that is the real disinfecting agent.

TABLE XXIII.

Silver nitrate. *B. paratyphosus*. 20° C. About 30,000,000 washed bacteria added to 5 c.c. AgNO_3 solution.

	Concentration AgNO_3		Time taken for disinfection = t	$\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0}$, where initial concentration, $C_0 = 5$, initial time, $t_0 = 0.75$
	Parts per 1000	Proportional nos. (0.17 per 1000 = 1) = C		
Exp. 24. 4. 07	0.85	5	0.75 min.	—
	0.17	1	1.5	0.10
	0.085	0.5	2.5	0.11
	0.017	0.1	5.5	0.15
	0.0085	0.05	22.5	0.10
	0.0017	0.01	56	0.16
	0.00085	0.005	140	0.14
	0.00017	0.001	more than 6.5 hrs.	—
Exp. 29. 3. 07	0.85	5	0.75	$C_0 = 5, t_0 = 0.75$
	0.017	0.1	6.5	0.15
	0.0085	0.05	21	0.11
	0.0017	0.01	69	0.15
	0.00085	0.005	122	0.16

* The values of the expression $\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0}$ are here all negative in sign, the time taken for disinfection increasing so slowly with decreasing concentration of AgNO_3 .

The above logarithmic formula was also found applicable to the case of disinfection of anthrax spores with mercuric chloride on further examination of the figures of Krönig and Paul (1897), already referred to. In their recent paper Madsen and Nyman (1907) give a table (p. 390), in which values of K , the velocity constant, are deduced for a series of Krönig and Paul's experiments. The velocity constant of any reaction being inversely proportional to the time taken for the completion of the whole or a definite fraction of the reaction, the above expression $\frac{1}{C_0 - C_n} \log \frac{C_n t_n}{C_0 t_0}$ may be also written $\frac{1}{C_0 - C_n} \log \frac{C_n K_0}{C_0 K_n}$.

In Table XXIV are given the values of K (from Madsen and Nyman) corresponding to different concentrations of mercuric chloride. Numbers expressing the corresponding concentrations of Hg^{++} ions were again deduced from the results of Luther and Kahlenberg; fortunately there was no need of extrapolation in this case. In column 4 are given values of the expression $\frac{1}{C_0 - C_n} \log \frac{C_n K_0}{C_0 K_n}$, numbers representing concentration of Hg^{++} ions being inserted for those of HgCl_2 , and very good constancy is maintained.

TABLE XXIV.

Mercuric chloride. Anthrax spores. 18°C . Krönig and Paul's experiments.

Parts HgCl_2 per 1000	Value of velocity constant $K \left(= -\frac{1}{t_2 - t_1} \log \frac{n_1}{n_2} \right)$ (Madsen & Nyman)	Nos. expressing concentration of Hg^{++} ions (Luther & Kahlenberg)	$\frac{1}{C_0 - C_n} \log \frac{C_n K_0}{C_0 K_n}$, where initial concentration of Hg^{++} ions, $C_0 = 88.5$, initial value of K , K_0 $= 0.22$
16.9	0.22	88.5	- -
8.4	0.14	83.0	0.031
4.2	0.08	76.8	0.032
2.1	0.07	69.0	0.023
1.1	0.026	61.0	0.028

As regards the effect of concentration of disinfectant upon disinfection velocity, there is no longer the close analogy shown in Section I to exist between disinfection and a chemical process involving two reagents which conforms to the unimolecular type, owing to the presence of one constituent in large excess. Whereas in the latter instance a simple proportionality exists between reaction velocity and concentration of reagent, the relation between velocity of disinfection and concentration of disinfectant was found to be a logarithmic one. The range of concentrations studied was necessarily limited to the cases where the corresponding times of disinfection could be measured with accuracy; many of these concentrations were approaching the limit of efficient action, where any chemical process involved is possibly reversible. It is conceivable that a simple proportion might still have been found to exist with higher concentrations; such an investigation is experimentally impossible, owing to the very short time that would be occupied by the reaction. Against this view, however, one may cite the experiments of Krönig and Paul, with anthrax spores and more concentrated solutions of mercuric chloride, which are discussed in the preceding paragraph.

Summary of Section II.

1. When phenol is used as a disinfectant a logarithmic relation exists between its concentration and the time taken for disinfection (the expression $\frac{1}{t_n - t_0} \log \frac{C_n t_n}{C_0 t_0}$ remaining constant in value). This was found to be true in the disinfection both of *B. paratyphosus* and of *Staphyl. pyogenes aureus*.

2. The same relation exists in the case of an emulsified disinfectant and *B. paratyphosus*, but only within narrow limits of concentration.

3. With silver nitrate and *B. paratyphosus* the above logarithmic relation holds good. In the case of mercuric chloride it is also true, if, in place of concentration of mercuric chloride, numbers are inserted representing concentration of Hg^{++} ions. This is shown in the case both of anthrax spores and *B. paratyphosus*. These facts lend further confirmation to the theory that, in the case of disinfection by metallic salts, the metallic ion is the real disinfecting agent.

4. The relation, expressed in 1, between velocity of disinfection and concentration of disinfectant, forms a marked contrast to the simple proportionality obtaining in the case of a chemical reaction of the unimolecular type, with which otherwise (see Section I) disinfection shows a close analogy.

5. Very small traces of salts of the heavy metals were found to prove inhibitive to bacterial growth. Bacteria which had been immersed in such solutions were therefore treated with sulphides when making test cultures. Large excess of sulphide was found indispensable, and, in the case of mercuric chloride, this is probably needed for the splitting up of some compound between the metal and the substance of the bacterium, which will prevent all further growth, however great dilution with culture medium be employed. If, however, a large excess of sulphide is administered subsequently as an antidote, the bacterium may recover its vitality.

SECTION III. THE INFLUENCE OF TEMPERATURE UPON THE VELOCITY OF DISINFECTION.

Robert Koch (1881) showed that the disinfection of anthrax spores with carbolic acid vapour was much more quickly completed if the temperature were raised; Henle (1889) showed the same to be true in the case of disinfection of *B. typhosus* with both phenol and creolin, and

Behring (1890) published the results of a few similar experiments made by Hünemann with mercuric chloride. Heider (1892) was the first to make a series of systematic experiments upon the subject, but as the temperatures employed lay chiefly between 40° C. and 80° C., it is probable that a mixed effect was being investigated, and that some at least of the disinfection was due to heat alone. Madsen and Nyman (1907) find that the velocity of disinfection of anthrax spores by mercuric chloride increases with rise of temperature in accordance with the well-known equation of Arrhenius, the increase being about 2·5-fold for a rise in temperature of 10° C. between the temperatures 25° C. and 45° C.

With the exception of a few experiments with anthrax spores, the work about to be described was all done with *B. paratyphosus*. The method generally employed was similar to that used in Section II, viz., a comparison of the times taken for (almost) complete disinfection at the different temperatures in question. In some cases, however, a comparison of the velocity constants of disinfection at various temperatures was made. This involves series of enumeration experiments, which are exceedingly laborious, and by means of which one can compare the reaction velocities corresponding to, at most, two temperatures. Some of the enumeration experiments in Section I were made simultaneously at different temperatures, and can be made use of in this particular.

Anthrax spores. In Section I (Tables I and II), the velocity constant during disinfection of anthrax spores has been shown to remain constant in value throughout the whole reaction, hence the values found at any two temperatures are perfectly comparable. Two experiments were made with 5% phenol at temperatures 20·2° C. and 33·3° C. respectively, and the velocity constant of disinfection at 20·2° C. (Table I) was 0·47, and at 33·3° C. (Table II) 0·44. Assuming that the velocity of disinfection of anthrax spores with phenol increases in a regular manner with rise of temperature, we get a coefficient for a rise in temperature of 10° C. equal to 5·5, a higher figure than that obtained by Madsen and Nyman for disinfection with mercuric chloride. These workers used the "garnet" method of Krönig and Paul (1897), and obtained their coefficient also by comparison of velocity constants.

B. paratyphosus. A direct comparison of velocity constants in the case of *B. paratyphosus* is much complicated by the fact that, as was shown in Section I, the value of these constants decreases progressively during the course of the disinfection. It is possible, on the other hand, to compare the average velocities of disinfection at different temperatures

by determining the times taken for the same number of exactly similar bacteria to be reduced in number by exactly the same amount. This was done by plotting the results of enumeration experiments at two different temperatures, and drawing smoothed curves. Similar points on these curves were subsequently read off and compared. It was essential, for justifiable comparison, that the material disinfected should be exactly similar in either case. The individuals in cultures of *B. paratyphosus* were shown in Section I to possess different resistances to the action of disinfectants. It was therefore necessary that the experiments at different temperatures¹ should be made with the same initial number of bacteria from the same culture, *i.e.*, exactly the same combination of the different resistances.

Two experiments in Section I fulfil these conditions (see Tables VI and VII). The same results are tabulated again in Table XXV *a* and *b* to show the effect of temperature upon disinfection, the times given being obtained from the smoothed curves in Figs. 6 and 7 respectively. The velocity of disinfection of *B. paratyphosus* with 6 per 1000 phenol

TABLE XXV.

Phenol, 6 per 1000. *B. paratyphosus*. 11° C. and 21° C.

No. of bacteria present in one standard drop of disinfection mixture	Time elapsing at 21° C. minutes	Time elapsing at 11° C. minutes	Relative increase in mean reaction velocity for a rise in temperature of 10° C.
Exp. 24. 7. 07. (a). (See Table VI and Fig. 6.)			
900	0	0	—
100	1·0	2·2	2·2
50	1·6	3·3	2·1
10	2·7	6·2	2·3
			Mean 2·2
Exp. 23. 7. 07. (b). (See Table VII and Fig. 7.)			
*	0	0	—
300	1·2	5	4·2
200	1·75	6·5	3·9
100	3	9·5	3·2
10	7·5	20	2·7
			Mean 3·3

* The initial number of organisms was not determined but was the same for experiments at either temperature.

¹ With an organism as sensitive as *B. paratyphosus* to temperatures above 45° C., the range over which experiments can be made is very limited. At temperatures above 20° C., even with dilute disinfectants, enumeration experiments have to be made with uncomfortable rapidity.

is shown to be increased in the one experiment 2.2 times, in the other 3.3 times, when the temperature is raised from 11° C. to 21° C.

The majority of the experiments were, however, made with the "end-point" method, described in Section II (p. 118), by means of which a comparatively large number of experiments at different temperatures could be made simultaneously.

A series of water baths was maintained at different convenient temperatures, tubes containing 5 c.c. disinfectant solution were placed in them, and inoculated in the usual way with five standard drops of a 24 hours' culture. The disinfection tubes were sampled from time to time, exactly as described in Section II, by withdrawing four standard drops (0.08 c.c.) and adding them to glucose or dulcitol broth; the end-points thus determined indicated a reduction of the total number of bacteria to less than 60.

To ascertain whether this less laborious "end-point" method could be substituted for that of comparing the velocity constants at different temperatures, simultaneous comparative experiments were made with exactly similar groups of bacteria. In the one case a comparison was made between the times elapsing at two temperatures before the total number of bacteria was reduced to less than 60, in the other the average reaction velocities at the same two temperatures were compared.

An "end-point" determination was made with a similar culture and similar number of organisms to those of Exp. *a*, Table XXV; unfortunately it was not made simultaneously. Phenol, 6 per 1000, was used, and at a temperature of 21° C. the total number of bacteria was reduced to less than 60 organisms in 6.75 minutes, being the mean of three concordant determinations; at 11° C. the time was 12.5 minutes, the same result being given by three experiments. The coefficient of increase in disinfection velocity for a rise of 10° C. is therefore 1.9. The figure obtained from the enumeration experiment was 2.2 (Table XXV, Exp. *a*).

To test this point further, experiments (Table XXVI) were made employing the two different methods simultaneously upon the same number of bacteria from the same culture. Some difficulty was experienced with the enumeration experiments at 21° C., and only one or two very rapid enumerations were possible before the number of surviving bacteria became too small to be counted with accuracy. The numbers inserted in Table XXVI, 3rd column, are those actually counted at 21° C. The times taken at 11° C., for a corresponding reduction in numbers, were derived from curves drawn through the points obtained by experiment. Figs. 17 *a* and 18 show the course of the latter part

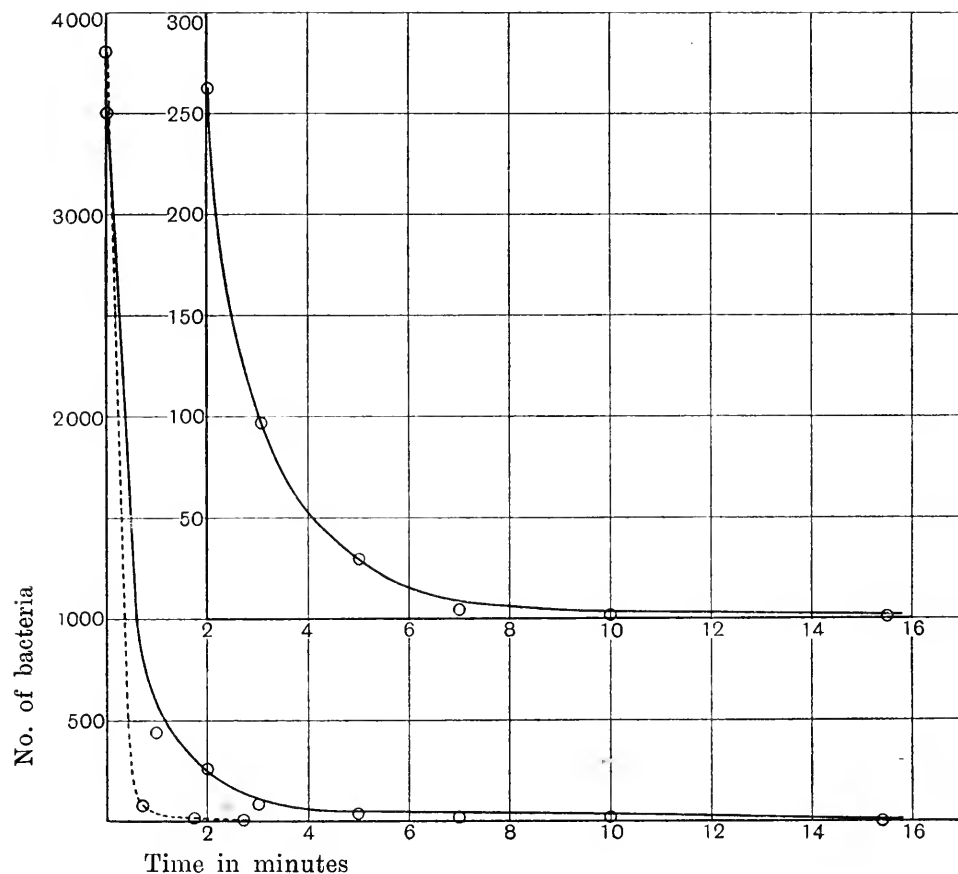


Fig. 17. (Exp. 24. 8. 07.) Disinfection of *B. paratyphosus* with phenol, 8 per 1000, at 11° C., continuous curve: at 21° C., dotted curve.

Fig. 16 a. (Exp. 24. 8. 07.) Showing course of the latter part of disinfection of *B. paratyphosus* with phenol, 8 per 1000, at 11° C. (Table XXVI, Exp. (a)).

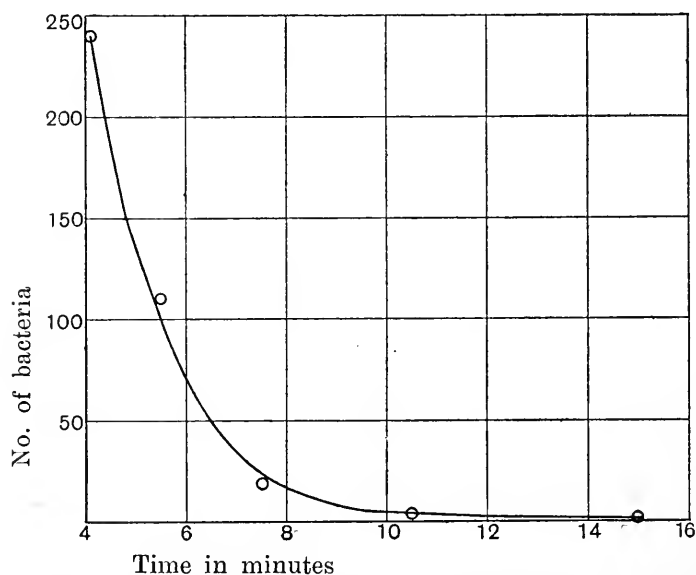


Fig. 18. (Exp. 27. 8. 07.) Showing course of the latter part of disinfection of *B. paratyphosus* with phenol, 8 per 1000, at 11° C. (Table XXVI, Exp. (b)).

of the disinfection at 11° C. in the case of Exps. (a) and (b) respectively, and were used to obtain the times given in Table XXVI, column 4. From this table it will be seen that the numbers obtained by means of the two methods showed close agreement, so that the subsequent employment of the "end-point" method was justified.

TABLE XXVI.

Phenol, 8 per 1000. *B. paratyphosus*. 11° C. and 21° C.

Method of experiment employed	Total no. of bacteria	No. of bacteria present in one standard drop of disinfection tube	Time elapsing at 21° C. minutes	Time elapsing at 11° C. minutes	Relative increase in mean reaction velocity for a rise in temperature of 10° C.
Exp. 24. 8. 07. (a).	950,000	3,650	0	0	—
"End-point"	60	—	6.5	24.5	3.8
"Enumeration"	—	79	*0.75	†3.35	4.4
Exp. 27. 8. 07. (b).	3,000,000	13,000	0	0	—
"End-point"	60	—	13.5	41	3.0
"Enumeration"	—	66	*1.75	†6.1	3.5
"	—	11.4	*3.25	†8.5	2.6

* Times of direct enumeration experiments.

† Times obtained from drawn curves, Figs. 17a and 18.

Experiments with B. paratyphosus, illustrating the effect of temperature upon reaction-velocity, in which the "end-point" method was employed.

In these experiments a much larger number of organisms was employed than were used in those in which a direct enumeration was made. The material disinfected was a 24 hours' culture. The increase in the number of organisms produced, when working with phenol, an unexpected rise in the value of the temperature coefficient. This variability according to the number of organisms used was found to be consistent, and the interpretation will be discussed later (p. 148).

1. Metallic Salts.

Mercuric chloride. The experiments were carried out exactly as those in Section II, with 20—40 million bacteria from a 24 hours' culture of *B. paratyphosus*. The precipitant used was sulphuretted hydrogen water, 0.15 c.c. of a saturated solution being added to each sub-culture tube containing 10 c.c. glucose broth.

TABLE XXVII.

Mercuric chloride, 1 in 1000. *B. paratyphosus*.

		Values of A $\left(= \frac{T_0 T_n}{T_0 - T_n} \log \frac{t_2}{t_1} \right)$, where initial absolute temperature $T_0 = 293$, and initial time $t_0 = 2.5$	
	Temperature degrees centigrade	Time taken for disinfection	
Exp. 9. 3. 07	42	< 0.5 min.	—
	29.7	< 0.5	—
	20	2.5	—
	12.6	4.5	2890
	7.9	6	2590
	0	41.5	4880
			Mean = 3420
			Initial temp. $T_0 = 293$, ,, time $t_0 = 1.5$
Exp. 12. 3. 07	26.8	1.5	—
	20	1.5	—
	13.2	3.5	4540
	7.2	11.5	5670
			Mean = 5100

* These, and all subsequent values of this expression, are calculated with Briggs' logarithms.

TABLE XXVIII.

Mercuric chloride, 1 in 10,000. *B. paratyphosus*.

		Values of A $\left(= \frac{T_0 T_n}{T_0 - T_n} \log \frac{t_n}{t_0} \right)$, where initial absolute temperature $T_0 = 314.2$, initial time $t_0 = 1.5$	
	Temperature degrees centigrade	Time taken for disinfection	
Exp. 14. 3. 07	41.2	1.5 min.	—
	29.9	2.5	*
	19.8	21	4930
	11.1	65	4850
	6.7	41	3690
	0	124	3990
			Mean = 4360
			$T_0 = 314.6$, $t_0 = 0.75$
Exp. 6. 3. 07	41.6	0.75	—
	30.7	2.5	4580
	19.8	11.5	5010
	13.9	36	5480
	6.8	50	4610
	0	101	4390
			Mean = 4810

* Figure is not concordant, probably due to inaccuracy in measuring the short intervals of time.

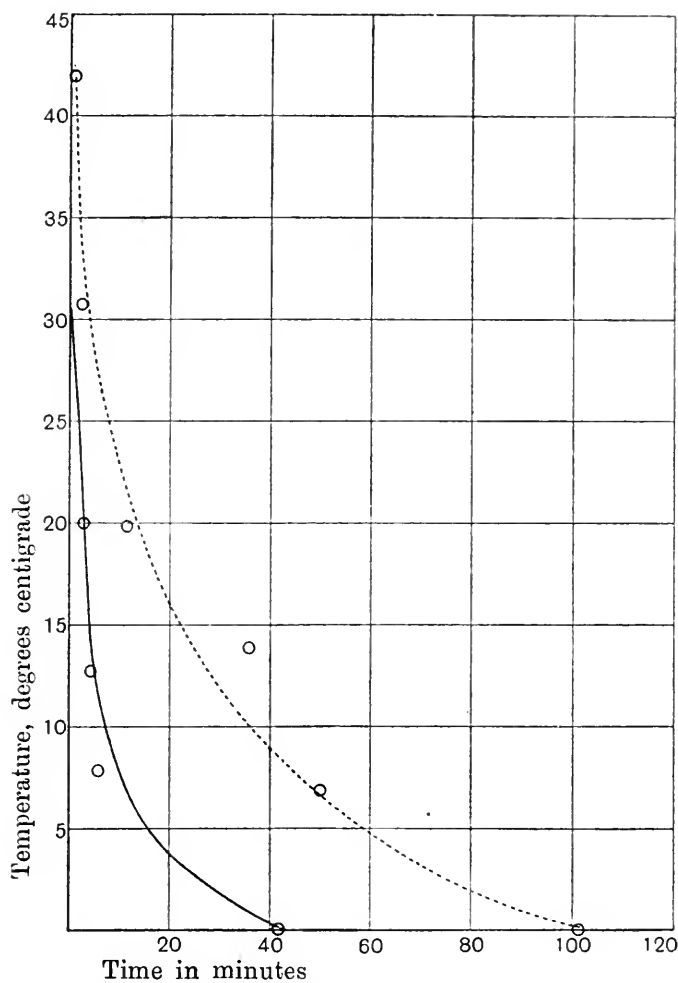


Fig. 19. Influence of temperature upon the time taken for disinfection of *B. paratyphosus* with mercuric chloride. Continuous curve, HgCl_2 1 in 1000 (Table XXVII, Exp. 9. 3. 07): dotted curve, HgCl_2 0.1 in 1000 (Table XXVIII, Exp. 6. 3. 07).

TABLE XXIX.

Mercuric chloride. *B. paratyphosus*.

	Concentration of mercuric chloride parts per 1000	Temperature degrees centigrade	Time taken for disinfection (derived from curve in Fig. 19) minutes	Relative increase in mean reaction velocity for a rise in temperature of 10° C.
Exp. 9. 3. 07, Fig. 19	1·0	25	1·2	3·3 2·6 4·0 5·8
		20	2·7	
		15	4	
		10	7	
		5	16	
		0	41	
Mean value = 3·9				
Exp. 6. 3. 07, Fig. 19	0·1	40	1·2	3·3 3·4 2·8 2·7
		30	4·0	
		20	13·5	
		10	38	
		0	102	
		Mean value = 3·0		

The results of two experiments with HgCl_2 1 in 1000 are given in Table XXVII, and of two experiments with HgCl_2 1 in 10,000 in Table XXVIII. Fig. 19 (abscissae = time taken for disinfection: ordinates = temperature of disinfection) shows the results of one set of experiments with either concentration, and the points found by experiment are seen to lie on or near to continuous curves, the velocity of disinfection increasing with rise of temperature in a very orderly manner (see Table XXIX).

Silver nitrate. An emulsion of washed bacteria was used as material to be disinfected in place of the usual drops from the broth culture, and dilutions were so arranged (see p. 129) that each experiment dealt with the disinfection of about 20—40 million bacteria.

The results of experiments with two concentrations of silver nitrate are given in Table XXX, and shown graphically in Fig. 20.

TABLE XXX.

Silver nitrate. <i>B. paratyphosus</i> .			
Concentration AgNO_3 parts per 1000	Temperature degrees centigrade	Time taken for disinfection	Values of A $\left(= \frac{T_0 T_n}{T_0 - T_n} \log \frac{t_n}{t_0} \right)$, where initial absolute temperature $T_0 = 313.2$ and initial time $t_0 = 0.67$
Exp. 25. 4. 07 0.017	40.2	0.67 min.	—
	30.7	2.6	5900
	20	13.5	5930
	12.25	17.5	4530
			Mean = 5450
			$T_0 = 314.1, t_0 = 4.5$
Exp. 21. 6. 07 0.0017	41.1	4.5	—
	33.3	35	*
	20.1	72.5	5280
	16.3	167.5	5740
	1.8	329	4090
			Mean = 5040

* Figure obtained was not concordant.

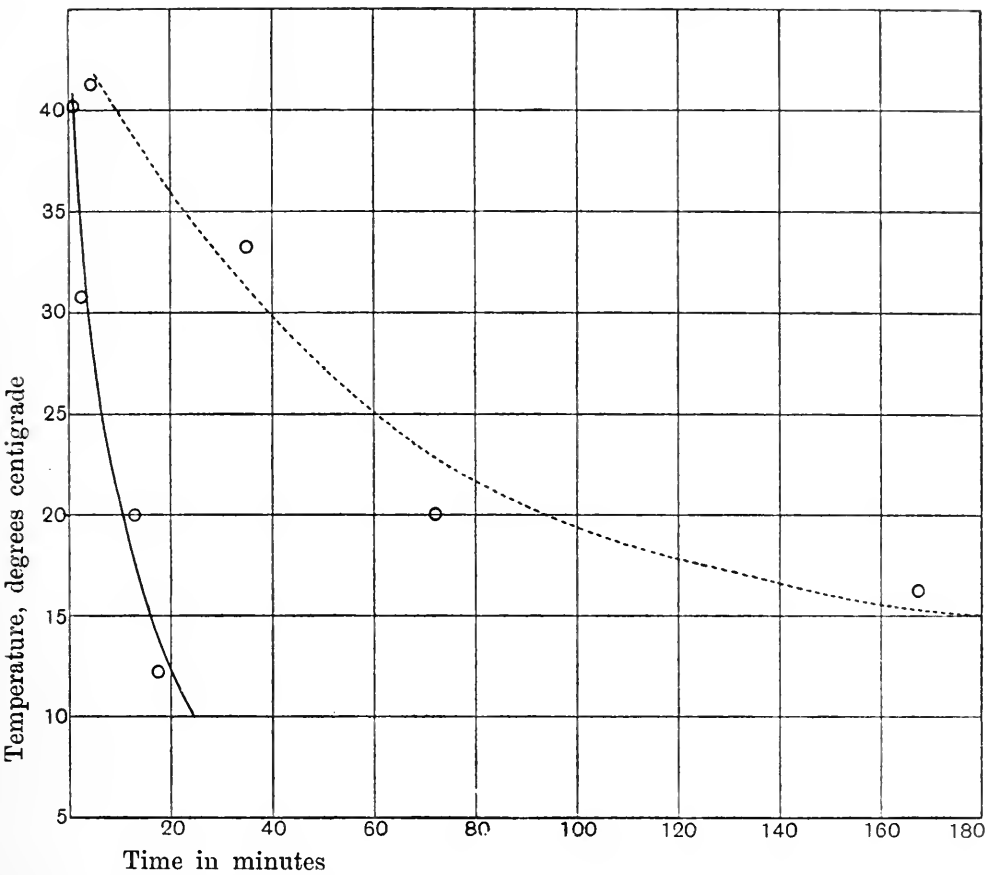


Fig. 20. Influence of temperature upon the time taken for disinfection of *B. paratyphosus* with silver nitrate. Continuous curve, AgNO₃ 0.017 per 1000 (Table XXX, Exp. 25. 4. 07); dotted curve, AgNO₃ 0.0017 per 1000 (Table XXX, Exp. 21. 6. 07).

TABLE XXXI.

Silver nitrate. *B. paratyphosus*.

	Concentration of AgNO ₃ parts per 1000	Temperature degrees centigrade	Time taken for disinfection (derived from curves in Fig. 20) minutes	Relative increase in mean reaction velocity for a rise in temperature of 10° C.
Exp. 25. 4. 07, Fig. 20	·017	40	1·2	3·3 2·9 2·3
		30	4	
		20	10·7	
		10	24·5	
				Mean value=2·8
Exp. 26. 1. 07, Fig. 20	·0017	40	9·7	3·9 2·6 2·4 3·0
		35	23·5	
		30	39	
		25	60·5	
		20	94·5	
		15	180	
				Mean value=3·0

The apparently consistent effect of rise of temperature upon the velocity of disinfection points again to a close analogy with the ordinary chemical reaction. The formula of Arrhenius, derived from the more complicated expression of Van t' Hoff, has been found applicable to many chemical reactions, viz., $A = \frac{T_0 T_n}{T_0 - T_n} \cdot \log \frac{K_0}{K_n}$, where A is a constant, and K_0 and K_n are the velocity constants of the reaction in question corresponding to the absolute temperatures T_0 and T_n respectively. Since the time taken for completion of a reaction may be considered as inversely proportional to the velocity of the reaction, one may rewrite the equation thus:— $A = \frac{T_0 T_n}{T_0 - T_n} \log \frac{t_n}{t_0}$, where t_n and t_0 are the times taken to complete¹ the reaction at absolute temperatures T_n and T_0 respectively.

This formula, found by Madsen and Nyman (1907) to apply to the disinfection of anthrax spores with mercuric chloride, is also applicable to disinfection of *B. paratyphosus* with both mercuric chloride and silver nitrate. Values of A , calculated from the results of experiment, are inserted in Tables XXVII, XXVIII and XXX, and are found to be approximately constant. A certain amount of discrepancy does exist, but difficulties doubtless arise from the small amount of inhibition, which cannot be avoided when working with metallic salts.

The increase of reaction velocity with rise of temperature is conveniently expressed as the relative increase per 10° C. rise in temperature. From Figs. 19 and 20 (which like all the subsequent curves in this section were constructed by drawing smoothed curves to include as nearly as possible all the points determined by experiment), numbers were obtained giving a series of values for this temperature coefficient in the case of disinfection by mercuric chloride and silver nitrate. These figures are given in Tables XXIX and XXXI respectively. Values of 2·8 and 3·0 were obtained for two concentrations of silver nitrate, and 3·9 and 3·0 were the values obtained in the case of two different concentrations of mercuric chloride, figures of the same order as those obtained for ordinary chemical reactions.

2. Phenol.

In Tables XXXII, XXXIII, XXXIV and XXXV is shown the

¹ In theory the reaction is completed only after an infinite time. In this instance the reaction is considered at an end, when less than 60 individual bacteria remain undisinfected, i.e., about $\frac{1}{50000}$ of the total number.

TABLE XXXII.

Phenol, 12.5 per 1000. *B. paratyphosus*.

	Temperature degrees centigrade	Time taken for disinfection	Value of $A \left(= \frac{T_0 T_n}{T_0 - T_n} \log \frac{t_n}{t_0} \right)$, where initial abs. temp. $T_0 = 29.4$ and initial time $t_0 = 2.5$
Exp. 25. 10. 06	21	2.5 minutes	—
	14.6	8	*
	2.6	216	8530
	0	460	8640
	- 3	more than 8.25 hours	—
			Mean = 8580

* Figure obtained was not concordant.

TABLE XXXIII.

Phenol, 10 per 1000. *B. paratyphosus*.

	Temperature degrees centigrade	Time taken for disinfection	Value of $A \left(= \frac{T_0 T_n}{T_0 - T_n} \log \frac{t_n}{t_0} \right)$, where initial abs. temp. $T_0 = 303.5$ and initial time $t_0 = 1.5$
Exp. 24. 9. 06	30.5	1.5 minute	—
	27.5	3.5 minutes	*
	21.3	9	8090
	16.5	27	7870
Exp. 26. 9. 06	15.8	23.5	7130
	6	226	7530
Exp. 25. 10. 06	0	17.5 hours	7910
			Mean = 7710

* Figure obtained was not concordant.

TABLE XXXIV.

Phenol, 8 per 1000. *B. paratyphosus*.

	Temperature degrees centigrade	Time taken for disinfection	Value of $A \left(= \frac{T_0 T_n}{T_0 - T_n} \log \frac{t_n}{t_0} \right)$, where initial abs. temp. $T_0 = 306.6$ and initial time $t_0 = 3.5$
Exp. 25. 9. 06	42.7	< 1 minute	—
	33.6	3.5 minutes	—
	29	9	8250
	21	47.5	8100
	16.3	94	7330
			Mean = 7890
Exp. 27. 9. 06	21	52.5	—
	16	101	—
	3	more than 23 hours	—

influence of temperature upon disinfection of *B. paratyphosus* in the case of four different concentrations of phenol. The same results are shown graphically in Fig. 21, where the four curves corresponding to the four different concentrations are all seen to be similar in form. The curves have a different slope from those expressing effect of temperature upon disinfection with metallic salts, the influence of temperature being here very much greater. The equation of Arrhenius was, however, applicable to this instance also; values of the expression $\frac{T_0 T_n}{T_0 - T_n} \log \frac{t_n}{t_0}$ are given in Tables XXXII—XXXV, and remain very constant. The

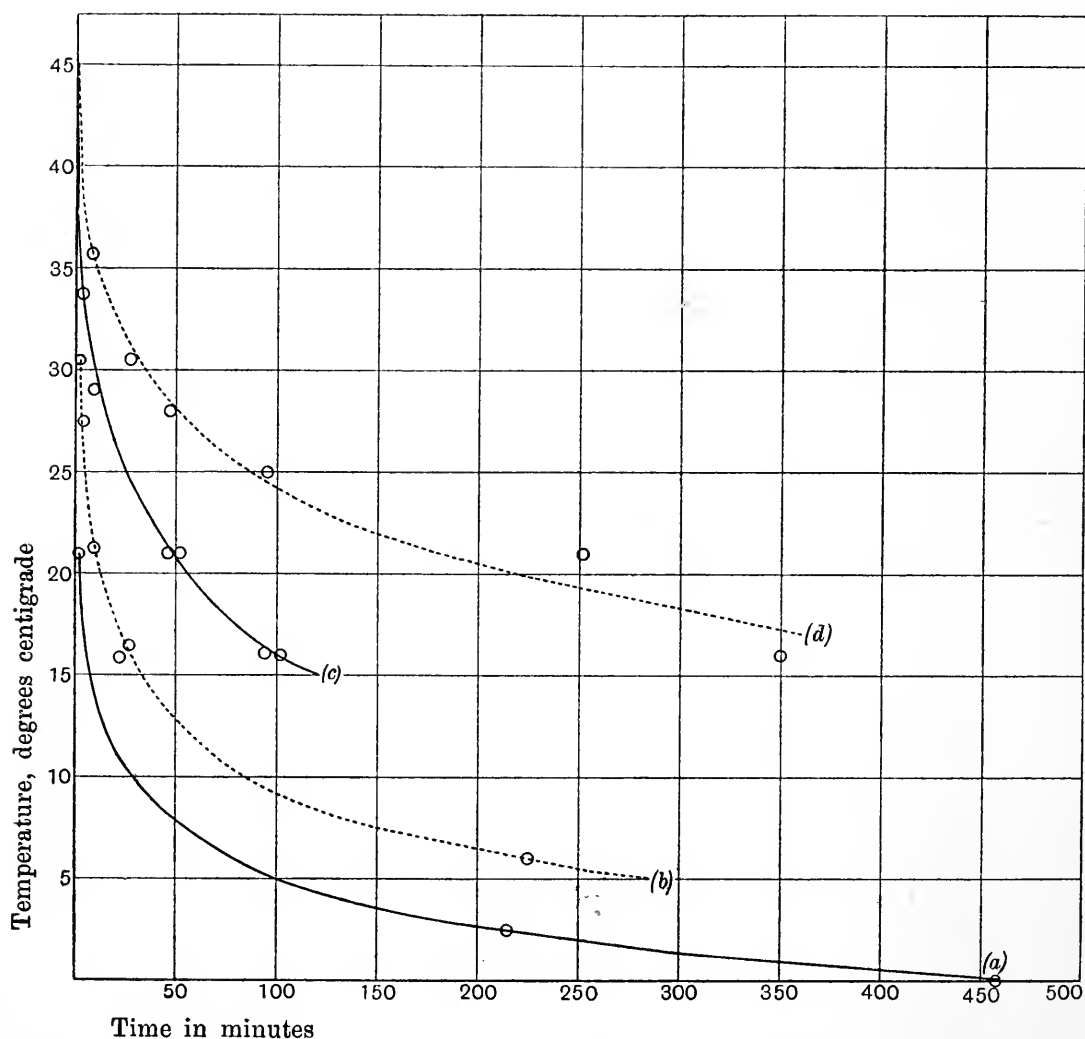


Fig. 21. Influence of temperature upon the time taken for disinfection of *B. paratyphosus* with phenol. Curve (a), phenol 12.5 per 1000 (Table XXXII, Exp. 25. 10. 06): curve (b), phenol 10 per 1000 (Table XXXIII, Exp. 24. 9. 06 and 26. 9. 06): curve (c), phenol 8 per 1000 (Table XXXIV, Exp. 25. 9. 06 and 27. 9. 06): curve (d), phenol 6 per 1000 (Table XXXV, Exp. 27. 9. 06 and 1. 10. 06).

TABLE XXXV.

Phenol, 6 per 1000. *B. paratyphosus*.

	Temperature degrees centigrade	Time taken for disinfection	Value of $A \left(= \frac{T_0 T_n}{T_0 - T_n} \log \frac{t_n}{t_0} \right)$, where initial abs. temp. $T_0 = 308.7$ and initial time $t_0 = 9$
Exp. 27.9.07	44	< 1 minute	—
	35.7	9 minutes	—
	30.5	27.5	8650
Exp. 1.10.06	28	47.5	8660
	25	95	8750
	21	251	8890
	16	350	7180
	6	more than 10 hours	—
			Mean = 8430
Phenol, 3 per 1000.			
Exp. 1.10.06	43	131 minutes	—
Phenol, 1.2 per 1000.			
	44	more than 24 hours	—

TABLE XXXVI.

Phenol. *B. paratyphosus*.

	Concentration of phenol parts per 1000	Temperature degrees centigrade	Time taken for disinfection (derived from curves in Fig. 21) minutes	Relative increase in reaction velocity for a rise in temperature of 10° C.
Fig. 21, curve <i>a</i>	12.5	20	2	14.0 14.3 16.4
		15	7	
		10	28	
		5	100	
		0	460	
		Mean value=14.9		
Fig. 21, curve <i>b</i>	10	30	2	6.0 8.1 7.1 8.8
		25	4	
		20	12	
		15	32.5	
		10	85	
		5	285	
		Mean value=7.5		
Fig. 21, curve <i>c</i>	8	35	3	8.3 6.2 4.8
		30	9	
		25	25	
		20	56	
		15	120	
		Mean value=6.2		
Fig. 21, curve <i>d</i>	6	35	11	7.9 6.5
		30	34	
		25	87	
		20	220	
		Mean value=7.2		

discrepancies occurring in some cases at the higher temperatures are doubtless due to the impossibility of measuring with accuracy the corresponding short intervals of time.

The temperature coefficients of the reaction velocity for an exact difference of 10° C. are given in Table XXXVI, where the times given in the 3rd column were obtained from the curves in Fig. 21. The value obtained for the highest phenol concentration appears to be abnormally high. In the case of the other concentrations the temperature coefficients are more nearly alike and the mean value, 7, is about twice as large as that obtained for disinfection with metallic salts.

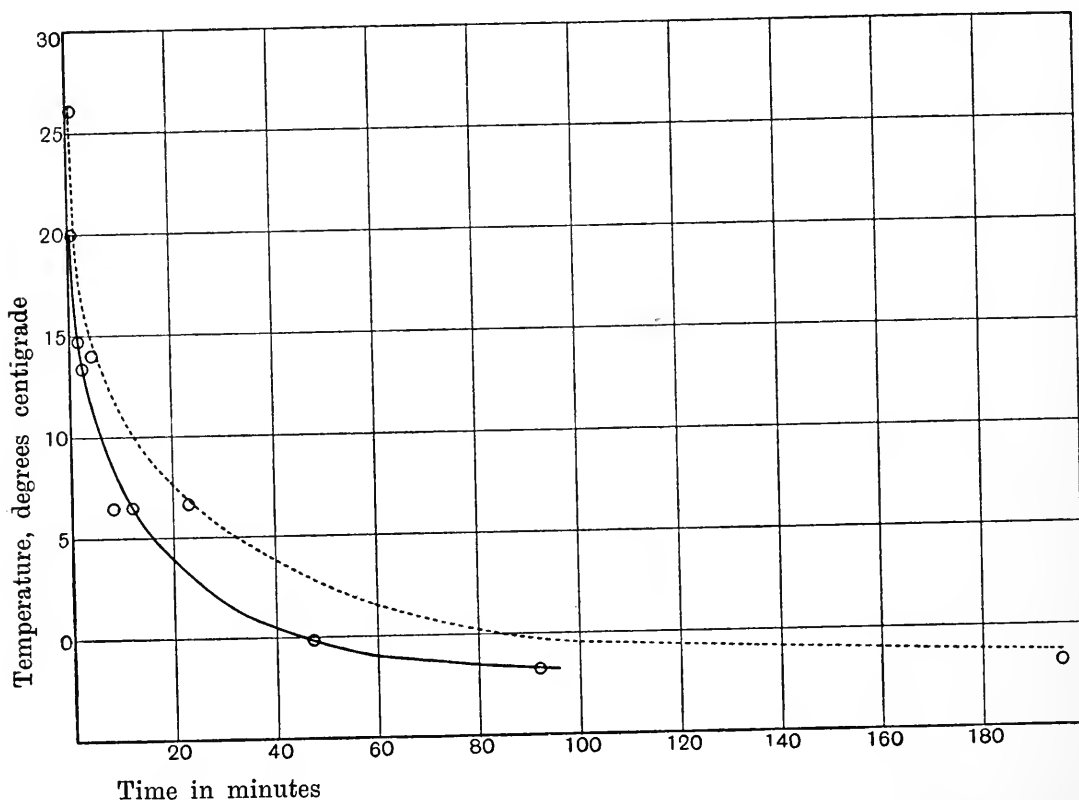


Fig. 22. Influence of temperature upon the disinfection of *B. paratyphosus* with disinfectant "A." Continuous curve, "A" 10 per 10,000 (Table XXXVII, Exp. 24. 1. 07 and 26. 1. 07); dotted curve, "A" 8 per 10,000 (Table XXXVIII, Exp. 2. 2. 07).

3. Disinfectant "A."

With regard to the effect of temperature, the disinfectant "A" falls into the same category as phenol. The results of five experiments with two different concentrations of "A" are given in Tables XXXVII and XXXVIII. The formula of Arrhenius was found applicable in this instance also, the expression $\frac{T_0}{T_0 - T_n} \log \frac{t_n}{t_0}$ remaining constant in value.

TABLE XXXVII.

Disinfectant "A," 10 per 10,000. *B. paratyphosus*.

	Temperature degrees centigrade	Time taken for disinfection minutes	Value of $A \left(= \frac{T_0 T_n}{T_0 - T_n} \log \frac{t_n}{t_0} \right)$, where initial abs. temp. $T_0 = 293$ and initial time $t_0 = 0.75$
Exp. 24. 1. 07	20	0.75	—
	13.4	2.6	6870
	6.7	12	7250
	- 0.3	47.5	7090
			Mean = 7070
			$T_0 = 293, t_0 = 0.87$
Exp. 19. 1. 07	20	0.87	—
	7.9	15	9010
	- 1.3	85	7440
			Mean = 8220
			$T_0 = 293, t_0 = 1.25$
Exp. 26. 1. 07	20	1.25	—
	14.7	1.5	—
	6.6	8.5	7830
	- 1.7	92	8710
			Mean = 8270

TABLE XXXVIII.

Disinfectant "A," 8 per 10,000. *B. paratyphosus*.

	Temperature degrees centigrade	Time taken for disinfection minutes	Value of $A \left(= \frac{T_0 T_n}{T_0 - T_n} \log \frac{t_n}{t_0} \right)$, where initial abs. temp. $T_0 = 292.9$ and initial time $t_0 = 6$
Exp. 29. 1. 07	19.9	6	—
	14	18.5	6970
	6	140	7860
	- 0.1	200	6080
			Mean = 6970
			$T_0 = 293.3, t_0 = 0.75$
Exp. 2. 2. 07	26.1	0.75	—
	20.3	0.75	—
	14	4.5	10400
	6.6	23.25	8920
	- 1.7	> 171	—
			Mean = 9660

Disinfection

The mean reaction velocity (see Fig. 22 and Table XXXIX) was found to increase 7 to 8-fold for a rise in temperature of 10° C., a figure approximating in value to that found for phenol, and again about twice as great as the number obtained in the case of disinfection by metallic salts.

Disinfection by phenol and the disinfectant "A" being thus equally influenced by temperature is evidence of the similarity of the actual process in the case of the two disinfectants.

TABLE XXXIX.

Disinfectant "A." *B. paratyphosus*.

Concentration of "A" parts per 10,000	Temperature degrees centigrade	Time taken for disinfection (derived from curves in Fig. 22) minutes	Relative increase in mean reaction velocity for a rise in temperature of 10° C.
10	20	0.8	6.9
	15	1.5	
	10	5.5	
	5	16	
	0	45	
			10.7
			8.2
			Mean value=8.6
8	25	0.7	6.1
	20	1.5	
	15	4.3	
	10	12.5	
	5	32.5	
	0	85	7.6
			6.8
			Mean value=7.1

This high temperature coefficient in the case of phenol was surprising in view of the results obtained from the enumeration experiments given in detail at the beginning of the present section (Tables XXV and XXVI). In those experiments, the mean velocity of disinfection was found to increase two or three, or, at most, four times with a rise in temperature of 10° C. To explain the discrepancy by a criticism of the "end-point" method was not possible, because, as shown above, perfectly concordant results were obtained from parallel experiments using the two methods, the same lower coefficient being obtained in either case (Table XXVI). The only difference between the earlier enumeration experiments and those just described is in the number of bacteria disinfected. In the former case, thousands only of bacteria were taken, in the latter case 20—40 million bacteria were disinfected in each experiment. Simultaneous parallel experiments were therefore made with the "end-point"

method, using exactly similar organisms from the same culture, but varying the total number disinfected, and, to make the comparison of results easier, water baths were maintained at temperatures differing exactly 10°C . from one another. The results are given in Table XL, each separate experiment showing the result of two sets of determinations, made over the same range of temperature, and concerned with the disinfection of exactly similar material, the quantity only of bacteria being different in the two cases. It was found to be invariably true that where more bacteria were disinfected, the temperature coefficient was higher (Exps. I, II, III and IV, Table XL).

TABLE XL.

Phenol. *B. paratyphosus*.

	Concentration phenol parts per 1000	No. of organisms added (approximately)	Temp. degrees centigrade	Time of disinfection (reduction of nos. in Col. II to less than 60) minutes	Relative in- crease in mean reaction velocity for a rise in temperature of 10°C .
Exp. I, 28. 8. 07	10	(a) 1,000	21	0.75	3.3
		„	11	2.5	
		(b) 750,000	21	2.5	6.0
		„	11	15	
Exp. II, 2. 8. 07	8	(a) 6,600	21	2.75	8.2
		„	11	22.5	
		(b) 1,030,000	21	25	10.3
		„	11	257	
Exp. III, 31. 8. 07	8	(a) 440,000	21	4.5	6
		„	11	27.5	
		(b) 76,000,000	31	4.5	7.7
		„	21	34.5	
		„	11	> 283	> 8.2
Exp. IV, 2. 8. 07	6	(a) 6,600	31	3.5	4.3
		„	21	15	
		(b) 750,000	31	20	10.4
		„	21	208	

In Section I, it was shown that among the individuals of a 24 hours' culture of *B. paratyphosus*, there existed permanent differences in resistance to disinfection, and that the younger organisms possessed the greater resistance. It therefore seemed possible that, among other differences, there might also be a different temperature coefficient for disinfection of bacteria of different resistances. The greater the total number of bacteria the greater also will be the number of more resistant forms. In any "end-point" method the properties of the more resistant

individuals alone are investigated; if then the disinfection of the latter should have a higher temperature coefficient than that of the less resistant individuals, disinfection experiments made with a large number of bacteria would show a higher temperature coefficient than those made with comparatively few¹. For in this latter instance the presence of a few of the more resistant bacteria with higher temperature coefficient will not be made evident by the experimental method.

TABLE XLI.

Phenol. <i>B. paratyphosus</i> .					
Concentration of phenol parts per 1000	Nature of culture employed	No. of organisms added (approximately)	Temperature of disinfection degrees centigrade	Time of disinfection (reduction of nos. in Col. III to less than 60) minutes	Relative increase in mean reaction velocity for a rise in temperature of 10° C.
Exp. 6. 9. 07.					
8	24 hrs.' culture	187,000	21	2.25	5.5
	"	"	11	12.5	
	"	56,000,000	21	32.75	12.2
	"	"	11	401	
	Young culture	81,500	21	0.8	13.7
	3rd generation	"	11	11	
Exp. 3. 9. 07.					
6	24 hrs.' culture	110,000	31	3.2	5.5
	"	"	21	17.5	3.9
	"	"	11	67.5	
					Mean = 4.7
	"	16,000,000	41	1.75	7.8
	"	"	31	13.75	10.3
	"	"	21	14.1	
					Mean = 9.05
	Young culture	8,850	31	1.5	8.1
	3rd generation	"	21	12.25	6.8
	"	"	11	83	
					Mean = 7.45

Special experiments were made to investigate the effect of temperature upon the disinfection of young organisms which were shown to possess the higher temperature coefficient. The necessary cultures were obtained by repeatedly sub-culturing in broth after intervals of

¹ It seems probable that in the case of disinfection with metallic salts, no such difference exists; a comparatively low temperature coefficient was obtained when large numbers of bacteria were disinfected. Whether this coefficient would be reduced in this case also, when small numbers were disinfected, could only be ascertained by further experiment.

about 3 hours. For purposes of comparison, simultaneous experiments were made with 24 hours' cultures, themselves taken from the same stock culture as the series of young growths (see Table XLI). Two concentrations of phenol were employed, and, in both cases, it was found that, whereas in the case of bacteria from a 24 hours' culture the high temperature coefficient was given only when a large number was present, an equally high coefficient is shown when only a few younger individuals were being disinfected. This result, combined with the theoretical considerations in the foregoing paragraph, yields additional evidence that, in a culture of *B. paratyphosus* the younger organisms offer the greater resistance to disinfection.

The fact that the younger and more resistant individuals in a culture of *B. paratyphosus* become relatively less resistant if the temperature is raised, is of great practical importance, and suggests an additional advantage in using hot or even warm solutions when disinfecting. In the case of spore-bearing organisms, such as *B. anthracis*, there probably exists no such complication, for the velocity coefficient of disinfection at constant temperature is the same for all individuals and no such difference in resistance can be detected. The temperature coefficients will doubtless be the same also for all the organisms.

TABLE XLII.

Results of Ballner's experiments with saturated steam and anthrax spores.

Temperature degrees centigrade	Time taken for complete sterilisation minutes	Values of $A \left(= \frac{T_0 T_n}{T_0 - T_n} \log \frac{t_n}{t_0} \right)$, where initial abs. temp. $T_0 = 378.3$ and initial time $t_0 = 0.43$
105.3	0.43	—
104.5	0.66	—
103.3	0.75	16480
102.3	0.9	16110
101.2	1.14	14610
100.7	1.7	18390
99.7	2.5	19190
98.4	2.8	16570
97.45	3.16	15460
96.4	3.3	13890
95.2	4.5	14070
94.2	5	13200
93.3	9	15260
92.7	8.7	14330
91.2	14	14780
90.4	14.7	14150

Some interesting parallel experiments upon disinfection by heat should be mentioned here. Ballner (1902) measured the time taken to kill an approximately constant number of anthrax spores, using saturated steam at different temperatures, and obtained a valuable series of figures (Table XLII). The object of his investigation was a purely practical one, concerning laboratory sterilisation at high altitudes. The formula of Arrhenius was, however, applied to his figures, and will be found to fit them very well, when the error inseparable from the measurement of very short intervals of time is taken into account (see values of "A," Table XLII). Ballner's results are expressed graphically in Fig. 23, where a smooth curve is drawn to include as many experimental points as possible. The velocity of disinfection is seen to be increased about tenfold (see Table XLIII) for a rise in temperature of 10°C. , a very high temperature coefficient.

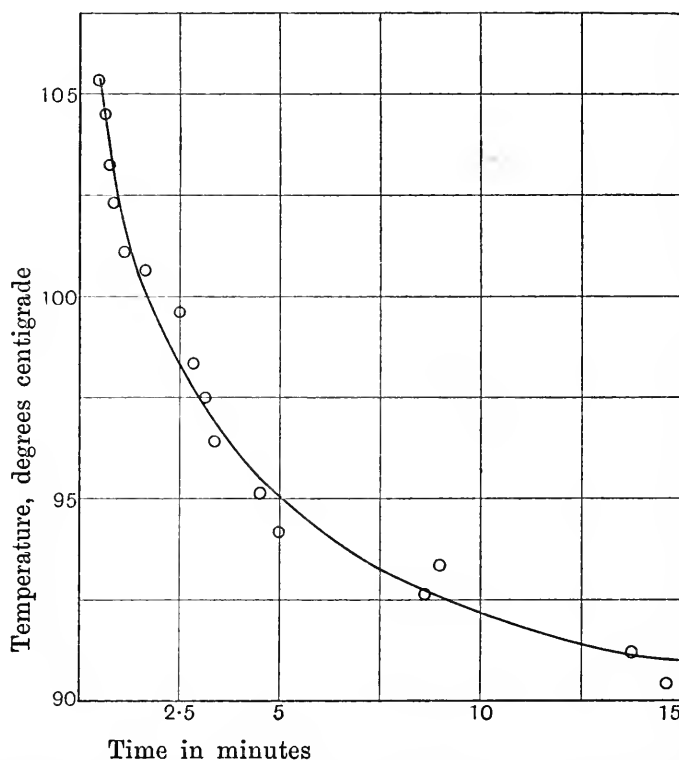


Fig. 23. Sterilisation of anthrax spores with saturated steam at different temperatures, from Ballner's results (see Table XLII).

Similar experiments by Meyer (1906) gave a much lower figure for the disinfection of the spores of *B. subtilis* and *B. robur*, the velocity of disinfection increasing only 4—5 times for 10°C. rise in temperature. Meyer examined his results for theoretical significance and found a logarithmic ratio to exist between the temperature of the experiment

and the time taken to kill. The series of temperatures formed terms of an arithmetical progression, while the corresponding times of disinfection (reciprocals of mean velocities of disinfection) were in geometrical progression. Meyer's relation may be expressed thus:

$$\frac{1}{t_1 - t_2} \log \frac{v_1}{v_2} = \text{constant},$$

where v_1 and v_2 are the reaction velocities of disinfection at temperatures t_1 and t_2 respectively.

The formula of Arrhenius has reference to the absolute temperatures involved, thus:

$$\frac{T_1 T_2}{T_1 - T_2} \log \frac{v_1}{v_2} = \text{constant},$$

where v_1 and v_2 are the reaction velocities of disinfection at absolute temperatures T_1 and T_2 respectively.

TABLE XLIII.

Results of Ballner's experiments derived from curve drawn in Figure 23.

Temperature degrees centigrade	Time of disinfection minutes	Relative increase in the mean velocity of disinfection for a rise in temperature of 10° C.
105	0.55	—
104	0.70	—
103	0.80	—
102	1.05	—
101	1.30	—
100	1.65	—
99	2.15	—
98	2.65	—
97	3.27	—
96	4.05	—
95	5.10	9.3
94	6.40	9.2
93	8.10	10.1
92	10.50	10.0
91	14.75	11.3

Mean = 10.0

The simpler relation is included in the expression of Arrhenius and applies perfectly to the results of Ballner, and also to all those obtained in the present investigation with chemical disinfectants. The values of both constants were calculated in the case of most experiments and equally concordant results were obtained. The question as to which formula should be applied could only be settled by means of experiments

over a much larger range of temperature, and this is impossible from the nature of the organism employed. For a range of temperature of only about 40° C., the value of the product T_1T_2 in Arrhenius' expression remains throughout very much the same in value, and the two formulae become almost identical. It might be instructive to make similar investigations with thermophilic bacteria.

Effect of Temperature upon Inhibition.

Behring (1890) showed that the influence of temperature upon inhibition was the exact opposite of that upon disinfection. He found that the growth of anthrax bacilli at room temperature was inhibited when 1 in 400,000 mercuric chloride was present; at 37° C. a concentration of 1 in 100,000 was necessary to inhibit growth. A similar effect was shown by Brooks (1906) in the case of fungi and various poisons.

These somewhat anomalous facts were confirmed by some experiments made during the present work with dilute phenol solutions and *B. paratyphosus*. The concentration of phenol had been reduced to 4 per 1000, in order to obtain a slower rate of disinfection, so that the effect of temperature might be more easily studied with enumeration experiments. The temperatures chosen were 20° C. and 30° C., and the very unexpected result was obtained that disinfection progressed more rapidly at the lower than at the higher temperature, the average value of the velocity constant being about 1·5—3·0 times as great at 20° C., as at 30° C. (Table XLIV).

TABLE XLIV.

Phenol, 4 per 1000. *B. paratyphosus*.

Temperature of experiment	Time elapsing minutes	Mean no. of bacteria present in one standard drop of disinfection mixture	K , assuming reaction to be in accordance with equation $-\frac{dn}{dt} = Kn$
Exp. 16. 7. 07 20° C.	1	685, taken as initial value of n	
	5	143	0·17
	11	175	0·16
	20	3	0·14
	1	661, taken as initial value of n	
	3	437	0·09
	5	238	0·11
	11	69	0·10
	22	0·66	0·14
Exp. 18. 7. 07 20° C.	1	459, taken as initial value of n	
	2	143	0·51
	3	27·3	0·61
	4	4·7	0·66
	5	1·7	0·56
	1	519, taken as initial value of n	
	2	464	0·05
	3	274	0·14
	4·4	124	0·18
	5·2	75	0·20
	7	28·5	0·21
	10	6·1	0·20
	15	0·96	0·19

In Section II (Table XVI), it is seen that a concentration of 4 per 1000 phenol took about 20 hours to complete disinfection when 6 per 1000 required only 4 hours. Phenol in the lower concentration may be said to be altering in character from that of a disinfectant to that of a solution able only to produce inhibition.

When bacteria are exposed to the action of weak antiseptics, it is evidently a very great point in their favour if the temperature should happen to be at or near that of their own growth optimum. In the case of strong solutions of disinfectants, the times involved are altogether too short for any effort after growth on the part of the bacteria to be apparent. But in the case of weak disinfectant solutions the organisms are able to make a struggle and are much assisted if the temperature is in the neighbourhood of their own growth optimum. Probably the results shown in Table XLIV would be reversed if the temperatures chosen were say 40° C. and 50° C. ; unfortunately such experiments would be useless because at 50° C. temperature itself becomes harmful to such an organism as *B. paratyphosus*.

Summary of Section III.

1. The reaction velocity of disinfection increases with rise of temperature in a manner similar to that of a chemical reaction. The formula of Arrhenius is applicable also to the case of disinfection, the expression $\frac{T_1 T_2}{T_1 - T_2} \log \frac{t_2}{t_1}$ (where t_1 and t_2 represent times taken for disinfection, reciprocals of mean reaction velocities, at absolute temperatures T_1 and T_2 respectively), remaining approximately constant in value.

2. The mean reaction velocity of disinfection with metallic salts increases 2—4-fold for a rise in temperature of 10° C. In the case of phenol and the disinfectant "A" the temperature coefficient is much higher, usually between 7 and 8. These experiments were made with about 20—40 million bacteria from a 24 hours' culture of *B. paratyphosus*.

3. The value of the temperature coefficient for disinfection of *B. paratyphosus* with phenol was found to vary with the number of individuals disinfected. The younger, and more resistant, individuals possess a higher temperature coefficient than the less resistant forms. The value of the coefficient varied from 2 to 10, according to the nature of the bacteria used for the experiment.

4. Inhibition is also influenced by temperature, but apparently in a different manner, being lessened or increased according as the particular temperature is near to or remote from that of optimum growth for the organism used.

GENERAL SUMMARY.

1. A very complete analogy exists between a chemical reaction and the process of disinfection, one reagent being represented by the disinfectant, and the second by the protoplasm of the bacterium.

2. Three classes of disinfectants were studied, (a) metallic salts (HgCl_2 and AgNO_3), (b) phenol, and (c) emulsified disinfectants (disinfectant "A"). *B. paratyphosus* and spores of *B. anthracis* were chosen as types of vegetative and spore-bearing organisms respectively.

3. In the case of anthrax spores, the disinfection process proceeds in obedience to the well-known equation for a unimolecular reaction, if numbers expressing "concentration of reacting substance" are replaced by "numbers of surviving bacteria."

4. Experiments with *B. paratyphosus* show a departure from the simple law owing to permanent differences in resistance to disinfectants among the individual organisms. The younger bacteria were proved to be the more resistant.

5. The process of disinfection is influenced by temperature in an orderly manner, and the well-known equation of Arrhenius can be applied.

(a) Disinfection of *B. paratyphosus* by metallic salts is influenced by temperature to about the same degree as most chemical reactions, the reaction velocity being increased about three-fold for a rise in temperature of 10°C .

(b) For disinfection of *B. paratyphosus* by phenol and the disinfectant "A" there was a much higher temperature coefficient, viz., seven to eight. In the case of phenol the effect of temperature was again found to be complicated by the want of uniformity among the individual bacteria. Disinfection of the younger, more resistant bacteria, was found to possess a higher temperature coefficient than that of the less resistant forms, the coefficient varying from ten to three, or two according to the age and number of the bacteria disinfecting.

6. It follows from (5) that there is a very great advantage in the use of warm solutions for practical disinfection.

7. Experiments, made with varying concentrations of disinfectant, and using similar groups of bacteria from cultures of *B. paratyphosus*, showed a definite logarithmic relation, between the concentration of disinfectant and the mean reaction velocity of disinfection, to exist in the case of phenol and the disinfectant "A."

8. In the case of silver nitrate, the same relation existed, but, in the case of mercuric chloride, numbers representing concentration of the salt had to be replaced by those representing concentration of the metallic ion. This confirms the theory that in disinfection with metallic salts the metallic ion is the real disinfecting agent.

9. This logarithmic relation is surprising in view of the simple proportionality existing in the case of chemical processes running the course of a unimolecular reaction, with which disinfection shows a close analogy.

10. Some evidence was obtained that, in disinfection with mercuric chloride, a toxic compound is formed between the metal and the substance of the bacterial cell. This compound prevents all further growth, but vitality can be restored by the administration of a large excess of soluble sulphide as an antidote.

I am glad to have this opportunity of expressing my great indebtedness to Dr C. J. Martin, at whose suggestion the work was undertaken, and who has helped me throughout, not only with most valuable advice, but also with practical assistance in many of the experiments.

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A very useful little work containing a good condensed description of modern methods. The names of the authors are a sufficient guarantee as to its character.

BOLDUAN, C. F. (1907). *Immune Sera*. 2nd ed. New York: John Wiley & Son. 154 pages. 10 figs. 19 × 12 cm. Price 6/-.

Comprises a brief exposition of our knowledge concerning the constitution and mode of action of antitoxins and other antibodies in serum. It had its origin in Wassermann's Monograph bearing a similar title (1904). New material has been added. The little book can be recommended to those desiring a brief summary of the subject.

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This little book is written especially for mayors and aldermen who are responsible for pure water supplies. The author describes the means now used by American cities to secure clean water. The illustrations are excellent, being photographs of the waterworks of various cities. They serve to illustrate principles and not waterworks of any special type. The book should prove very useful—not only to those for whom it is intended.

JONES, W. H. S. (1907). *Malaria. A neglected Factor in the History of Greece and Rome*. With an Introduction by R. Ross and a chapter by G. G. Ellett. Cambridge: Macmillan & Bowes. 107 pages. 19 × 13 cm.

A very interesting little work bringing evidence, gleaned from the writings of classical authors, regarding the existence of malaria in ancient Greece and Rome.

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This book constitutes the third edition of "Notter and Firth" (1896), revised and so largely rewritten by Lieut.-Col. R. H. Firth as to practically constitute a new work. The book is a standard work of the first order.

- PARKES, L. C. and KENWOOD, H. R. (1907). *Hygiene and Public Health*. (Third Edition.) London: H. K. Lewis. 620 pages. 95 figs. 22 × 14 cm.

This excellent treatise can be safely recommended to students of Hygiene and Public Health. Whilst new matter has been introduced the book is not unduly large, the size of the page being larger than in the previous editions.

- ROGERS, L. (1908). *Fevers in the Tropics*. London: Henry Frowde, Oxford University Press, and Hodder & Stoughton. 343 pages. 11 plates, 82 charts, 5 diagrams, 33 tables. 25 × 19 cm.

This important contribution to the literature of the fevers prevailing in the tropics will be welcomed by medical readers, since it contains much original matter collected by the author in India. It contains his Milroy Lectures on Kala-azar and sections on Yellow Fever and Sleeping Sickness, besides those dealing with typhoid, paratyphoid, spirochaetosis, Malta fever, amoebiasis, epidemic dropsy, malaria, dengue, plague, heatstroke and various unclassified fevers, and the methods of blood examination. All the illustrations are original and good.

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A semipopular brochure being mostly a collection of letters to the Press. Advocates measures against rats, etc.

REPORTS ON PLAGUE INVESTIGATIONS
IN INDIA.

ISSUED BY

THE ADVISORY COMMITTEE

APPOINTED BY THE SECRETARY OF STATE FOR INDIA, THE
ROYAL SOCIETY, AND THE LISTER INSTITUTE.

(Charts I—VII.)

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XXVI. THE PART PLAYED BY INSECTS IN THE EPIDEMIOLOGY OF PLAGUE.

By D. T. VERJBITSKI, M.D.

(*From the Laboratory of the Emperor Alexander I, and of the Imperial Institute of Experimental Medicine at St Petersburg.*)

INTRODUCTION TO TRANSLATION.

Dr Verjbitski's experiments were made in 1902—3 at Cronstadt and St Petersburg and the results were presented as a thesis for the degree of Doctor of Medicine to the University of St Petersburg in 1904. Owing to the fact that the thesis was written in the Russian language and not published in any scientific journal this important research has apparently been lost sight of, and has also for the most part escaped abstraction by the journals of Western Europe¹. In consequence it has not as far as we are aware been referred to by any of the workers on the subject since its presentation.

During the early part of last year the Committee succeeded in obtaining a copy of Dr Verjbitski's thesis which has been kindly translated for them by Mrs J. B. Leathes. Dr Verjbitski's experiments appeared to the Committee so admirably conceived and executed, and the results obtained by him so important in relation to the spread of the plague through the agency of insects, particularly fleas, that they communicated with the author requesting permission to publish an account of them as an appendix to one of the reports of the work of the Plague Commission.

*Dr Verjbitski's researches covered much of the ground subsequently traversed by the Commission, although the particular species of fleas worked with in the two instances were not identical. According to Verjbitski the common flea captured off rats in Cronstadt was *Typhlopsylla musculi*, the more usual host of which is, in other localities, the*

¹ The attention of the Committee was drawn to the work by a short abstract in the *Journal de Physiologie et de Pathologie générale*, vol. VIII. 1906, p. 387.

mouse. This flea was not found to bite human beings, so that the full epidemiological significance of Dr Verjbitski's work was not apparent.

In preparing Dr Verjbitski's papers for publication in English, some minor omissions and alterations have been considered advisable. The historical introduction has been omitted, as the literature on the subject received full treatment in these reports (*Journal of Hygiene*, vol. VI. p. 425). In the original thesis a very full and detailed account of the pathological findings at the post-mortem examinations of the various animals which succumbed to infection is given. In the translation this has usually been omitted and the facts resumed by some such statement as:—"the post-mortem presented a characteristic picture of plague infection." Further, in the original, the author, after having adequately presented the results of his experiments in tabular form, subsequently reiterated them at considerable length in the text. This, however, appeared in most cases to be unnecessary and in the translation the Tables have been usually allowed to speak for themselves. Otherwise the text has been as faithfully preserved as was consistent with the different formation of the two languages.

PART I. EXPERIMENTS WITH FLEAS.

In order to ascertain the part played by parasites in the spread of plague infection, I chose for my experiments fleas and bugs as being the parasites which chiefly frequent the human being. In my experiments with fleas, rats were employed in view of the established connection between plague epidemics amongst people and plague epidemics amongst rats.

Once the possibility of infection by the bites of fleas which are infected with plague microbes was proved, the transference of plague from rat to rat and from rat to man and *vice versa*, would easily explain both plague epidemics amongst rats and the connection between the latter and the plague epidemics amongst people.

The first questions which arose were:—did rat fleas bite men, and did human fleas bite rats? The flea which is mostly found on rats is *Typhlopsylla musculi*¹.

To answer these questions the following series of experiments were undertaken.

¹ Dr Verjbitski is presumably referring to his experience in Cronstadt. The commonest rat flea in most parts of Europe is *Ceratophyllus fasciatus*, and in India and most sub-tropical countries *Pulex cheopis* of Rothschild (see *Journal of Hygiene*, vol. VI. 1906, p. 483). Ed.

(1) Fleas were combed out of grey rats into a high cylinder and were gathered in a test tube, which was applied with its opening downwards to the wrist of a human being. In the majority of cases the fleas did not bite, but sometimes positive results were obtained, and careful examination of the fleas made it clear that these bites were not occasioned by rat fleas but by *Pulex canis*. In 40 experiments confined to *Typhlopsylla musculi* these fleas, even after lengthy previous starvation (from 10—40 hours), did not bite men.

(2) Human fleas, *P. irritans*, enclosed in test tubes were in the above manner applied to the shaved inner surface of the hind leg of a grey rat. Under these circumstances they invariably fed and the more readily the longer the fleas were allowed to starve before the experiment.

We have just mentioned that, besides ordinary rat fleas, *P. canis* was observed on grey rats; this circumstance made me turn my attention to fleas found on domestic animals and their relation to rats and man.

For these experiments I took dog and cat fleas, *P. canis* (*Ctenocephalus novemdentatus*) and *P. felis* (*Ctenocephalus enneodius*) as being those belonging to domestic animals which stand in most intimate relation to man. The macro- and microscopical appearances which I observed in these fleas (*P. irritans*, *P. canis*, and *P. felis*), corresponded entirely with the description of these parasites given by Professor Brandt and Neveu-Lemaire whose papers I had at my disposal whilst investigating the above species of fleas.

In order to make clear the relation of dog and cat fleas as occasional parasites of rats and man, I made the following experiment.

(3) Fleas collected from dogs and cats were starved for 10 hours, and allowed to bite both rat and man. In both cases a positive result was obtained; dog and cat fleas immediately attacked both man and rat leaving very clearly defined bites. Therefore *P. canis* and *P. felis* bite both man and rat, and the fact that they were found on rats shows that they had lived on the latter as casual parasites.

I have investigated whether *P. irritans*, *P. canis*, and *P. felis*, which were artificially brought into contact with rats, would continue to live on them, and if so for how long. This experiment was done in the following way:

(4) Fleas collected from cats and dogs were placed in high cylinders, 15 in each. There were 10 cylinders. Cylinders 1 to 5 contained cat-fleas; 6 to 10 dog-fleas. Into each cylinder a grey rat

was put. After half an hour all the rats were taken out, but no fleas were left in the cylinders. The rats were afterwards put into cages. After two days the fleas were combed out into glass pots from the rats 1 and 6, and the number of *P. canis* and *P. felis* was counted; after four days the same was done with rats 2 and 7; after six days with 3 and 8, and so on. The number of fleas obtained was probably smaller than that actually present, as it was not found possible to comb out all the fleas from the rats.

TABLE I.

Species of fleas	Numbers of rats	No. of fleas found on						
		1 day	4 days	6 days	8 days	10 days	12 days	14 days
<i>Pulex felis</i>	1	6	—	—	—	—	—	—
„	2	—	4	—	—	—	—	—
„	3	—	—	5	—	—	—	—
„	4	—	—	—	2	—	—	—
„	5	—	—	—	—	—	—	0
<i>Pulex canis</i>	6	5	—	—	—	—	—	—
„	7	—	7	—	—	—	—	—
„	8	—	—	4	—	—	—	—
„	9	—	—	—	—	—	1	—
„	10	—	—	—	—	—	—	0

Therefore, on the 8th—12th day from the beginning of the experiment it was still possible to find *P. canis* and *P. felis* on the rats, which indicates that these fleas can live on rats. The same experiment was repeated with *P. irritans*.

(5) Ten human fleas were put on each of two rats. After four days three were found on the first, and after eight days two fleas on the second. This proves that *P. irritans* can also live on the rat.

If under artificial conditions *P. irritans*, *P. canis*, and *P. felis*, can live on the rat it was interesting to ascertain if they lived there under natural conditions. Having at my disposal a number of grey rats for experiments, I combed the fleas out of them and determined their species. Altogether I looked over 300 rats, on 37 of which I found *P. canis* in addition to the ordinary *Typhlopsylla musculi*. The number on each rat fluctuated between one and four. The rest of the rats only harboured *Typhlopsylla musculi*; on none of them could I find *P. irritans* or *P. felis*.

From the above experiments I conclude :

1. The rat flea *Typhlopsylla musculi* does not bite men.
2. Human fleas bite rats.

3. Fleas of dogs and cats bite both men and rats.

4. Human fleas and fleas of dogs and cats can live as parasites on rats.

This latter circumstance, in connection with the fact that cat and dog fleas also attack man, makes it clear that under certain conditions transmission of these fleas from rat to man and *vice versa* is quite possible. Such favourable conditions appear for instance in the conditions surrounding the lives of the Kirguises (nomad race of Siberia), where frequent plague epidemics have been observed. People and domestic animals live together in dirty earth huts, with absence of ventilation, overcrowding, and insufficient light. The mats which cover the floor of these huts are teeming with fleas, partly *P. irritans*, but chiefly *P. canis*. If we take into consideration that during the winter the life of the Kirguise is spent on these mats, that they sit, eat, and sleep on them, it is easy to understand the possibility of these fleas indiscriminately living both on man and on the rats of which there is an abundance in these huts.

The investigations of Ogata, the German Commission, and others, have determined that fleas caught on dead rats, and rats ill with plague, may contain virulent plague microbes. My experiments always gave a positive result where the investigation concerned rat fleas which had been collected from dying or just dead rats, and also in the cases where human fleas and fleas of domestic animals had sucked the blood from a rat dying from plague. In every case I found plague microbes which corresponded in their virulence to the culture with which the animal had been inoculated. I carried out my experiments in the following way.

Experiment I.

Rat fleas collected from a rat which had died from plague, and *P. irritans*, *P. canis*, and *P. felis*, which had sucked the blood of a rat dying from plague, were crushed between two glasses and carefully smeared over the surface; these smears were fixed and stained with eosin and methylene blue. The microscopical examination showed blood corpuscles more or less altered according to the time since ingestion. Amongst these corpuscles were found single oval bi-polar staining bacilli which did not stain by Gram. Their number in most cases was not large, one, two or perhaps three in the field of vision. In these preparations one could also observe other forms of

bacilli, but more often cocci of different sizes. More than 100 investigations of this kind were made, and always with the same result.

In order to definitely establish the fact that the observed microbe was that of plague, it was necessary to cultivate it.

Experiment II.

Fleas of the species *Typhlopsylla musculi*, *P. irritans*, *P. canis*, and *P. felis* were allowed to suck the blood of a rat dying of plague and then crushed between two glasses. The glasses were afterwards immersed into tubes containing alkaline broth, which in their turn were put for 24 hours into the thermostat. After 24 hours, growth accompanied by opacity of the broth was observed. Microscopical examination showed that the culture was not pure. Sub-culture upon agar gave:

1. Round colonies resembling seeds with a festooned edge, which consisted sometimes of short bi-polar staining bacilli.

2. Yellow or white colonies more or less opaque which consisted either of cocci or of bacilli of different sizes.

No. 1 on all nutrient media gave a growth typical of plague. On inoculating rats, white mice and guinea pigs with this culture death invariably took place and the post-mortem presented a characteristic pathologico-anatomical picture of plague. The microscopical investigation of the growth on agar and in broth of both the blood and organs of these animals established the presence of plague bacilli in pure culture. Cultures obtained from the other colonies (No. 2) consisted either of cocci or of bacilli. Their inoculation had no effect on animals except occasional suppuration at the spot of inoculation. These microbes are commonly found in the intestine or on the surface of the flea's body.

Cultures of plague from fleas, which had sucked the blood of rats dying of plague, were also obtained in another way.

Experiment III.

Fleas collected from a rat dying of plague were put into a test tube with physiological salt solution, and were crushed by means of a glass rod and carefully mixed with the salt solution. The emulsion obtained in this way was used for inoculating rats. The result of these inoculations was not always the same and depended partly on the virulence of the culture which had killed the rat whose parasites these fleas had

been, and partly upon the number of crushed fleas used for inoculation. The more virulent the culture, the less fleas sufficed to bring about infection. As will be seen from the following table, in the beginning of the experiments when the rats on which the fleas fed were killed by very weak cultures, only those rats died which had been inoculated with an emulsion of 10 fleas. As the culture grew more virulent fewer fleas were required, and when the minimal lethal dose of the culture corresponded to $\frac{1}{500000}$ c.c. of a 24 hours' broth culture, the infection and death of a rat could be obtained by injecting subcutaneously the emulsion of one flea.

TABLE II.

Minimal lethal dose of the culture with which rats on which fleas had fed were inoculated	Number of fleas collected on rats which were crushed and inoculated into a fresh rat	Result on the fresh rat	Time of death
$\frac{1}{100}$ c.c. 24 hours broth culture	4	lived	
	6	lived	
	8	lived	
	10	died	110 hours
$\frac{1}{10000}$ c.c. 24 hours broth culture	2	lived	
	4	lived	
	6	died	104 hours
	8	died	98 hours
$\frac{1}{100000}$ c.c. 24 hours broth culture	1	lived	
	2	lived	
	3	died	101 hours
	4	died	119 hours
$\frac{1}{500000}$ c.c. 24 hours broth culture	1	died	100 hours
	1	lived	
	2	died	97 hours
	2	died	102 hours

The post-mortem examination of the rats killed in this way showed haemorrhagic infiltration at the place of inoculation, buboes, fatty liver and haemorrhages of the serous membranes. Cultures of the blood and organs on agar or in broth always gave a pure growth of plague bacilli. Analogous results were obtained when instead of rat fleas, *P. irritans*, *P. canis*, and *P. felis* were taken.

As it would seem not improbable that the fleas can under certain circumstances transmit plague infection, I next ascertained how long the plague microbe can live in the body of a flea, or rather, how long after it has entered the body of a flea it could still be detected. The necessary nourishment of the flea was in the meantime effected by feeding upon a healthy animal. The results of the following experiments serve to answer this question.

Experiment IV.

Rat fleas, and *P. irritans*, *P. canis*, and *P. felis* were placed each in separate test tubes and after previous starvation were allowed to bite rats dying from plague. The rat was put into a beaker, the inner surface of one of its hind legs was carefully shaved, and the opening of the test tube was carefully applied to it and inverted. Each test tube contained two or three fleas. After feeding the test tubes were reversed and closed with cotton wool and put into a cupboard at the temperature of from 16—18° C. On the following day one of each kind of flea was crushed between two sterilised glasses, one of which was afterwards fixed and stained for microscopical examination, while the second was used to obtain a broth culture.

The fleas remaining in the test tubes were in the same manner fed again on another, but this time a healthy rat: 24 hours after, one of each was again taken for both microscopical and cultural purposes, and the remaining fleas were again fed on another healthy rat, etc. Fifteen of each species of fleas were used for this experiment, and the investigation was continued for 13 days.

The subsequent history of the healthy rats upon which the fleas fed I shall reserve for future discussion, but the answer to the question how long the bacilli remain alive within the flea is given in Table III.

TABLE III.

On which day after allowing fleas to bite a rat dying of plague the microscopical and bacteriological investigation took place.

	1	2	3	4	5	6	7—15
Species of fleas	Microscopical investigation Culture	Microscopical investigation Culture	Microscopical investigation Culture	Microscopical investigation Culture	Microscopical investigation Culture	Microscopical investigation Culture	Microscopical investigation Culture
<i>Pulex irritans</i>	+ +	+ +	+ +	+ +	+ +	- +	- -
<i>Pulex canis</i>	+ +	+ +	+ +	+ +	+ +	- -	- -
<i>Pulex felis</i>	+ +	+ +	+ +	+ +	+ +	- -	- -
<i>Typhlopsylla musculi</i>	+ +	+ +	+ +	+ +	+ +	- +	- -

+ plague bacilli present, - plague bacilli not found.

As the plague microbe did not lose its vitality after five to six days in the body of the flea, it was interesting to determine how

this sojourn acted on its virulence, that is to say, whether the culture obtained from the plague microbes out of the fleas corresponded to the virulence of the culture with which the rat was infected. The following experiment was undertaken with a view to determining this point.

Experiment V.

Four rats were infected with cultures of different virulence:—

1. With cultures whose minimal lethal dose corresponded to $\frac{1}{100000}$ c.c. of 24 hours' broth culture.

2. With cultures whose minimal lethal dose corresponded to $\frac{1}{1000000}$ c.c.

3. With cultures whose minimal lethal dose corresponded to $\frac{1}{5000000}$ c.c.

4. With cultures whose minimal lethal dose corresponded to $\frac{1}{10000000}$ c.c.

When these rats were beginning to die, fleas were applied to them:—

To the first rat, specimens of *Typhlopsylla musculi*.

To the second rat, specimens of *P. irritans*.

To the third rat, specimens of *P. canis*.

To the fourth rat, specimens of *P. felis*.

TABLE IV.

The fleas had 4 days ago sucked the blood of a rat killed by a culture of which the minimal lethal dose was equal to	Species of flea from which the culture was obtained	Dose of this culture used (24 hours broth culture)	Nos. of rats	Results of experiments	Time of death
$\frac{1}{100000}$ c.c.	<i>Typhlopsylla musculi</i>	$\frac{1}{100000}$ c.c.	{ 1 2 3	{ — + +	{ — after 92 hrs. ,, 102 ,,
$\frac{1}{1000000}$ c.c.	<i>Pulex irritans</i>	$\frac{1}{1000000}$ c.c.	{ 1 2 3	{ — — +	{ — — after 110 hrs.
$\frac{1}{5000000}$ c.c.	<i>Pulex canis</i>	$\frac{1}{5000000}$ c.c.	{ 1 2 3	{ + + —	{ after 91 hrs. ,, 115 ,, —
$\frac{1}{10000000}$ c.c.	<i>Pulex felis</i>	$\frac{1}{10000000}$ c.c.	{ 1 2 3	{ + — +	{ after 112 hrs. — after 117 hrs.

+ rat died, — rat lived.

After this, these fleas were fed during four days on healthy animals, and on the fifth day one of each was crushed between two glasses, the latter were immersed into test tubes of broth and placed in the thermo-

stat. After 24 hours the organism was obtained by sub-culture. In this way four plague cultures were obtained from the fleas, and the 24 hours' cultures in broth were tested with regard to virulence. The results are given in Table IV.

We see therefore that the plague microbe had retained its vitality, and that its virulence did not practically differ from the virulence of the culture with which the animal had been infected.

Experiment VI.

Six of each of the four species of fleas were applied to a rat dying of plague. Two of each kind were immediately afterwards drowned in a test tube containing broth. After two days two of each species were again treated in the same manner. The remaining fleas received the same treatment after four days, although in the meantime they had died, as during absolute starvation the fleas of the above kinds do not live more than two to two and a half days. All these test tubes were put for 24 hours into the thermostat. Only in some cases could plague bacilli be obtained by plating. Out of the first eight cultures only four gave a positive result, viz.

Two of the test tubes which contained *P. canis*.

One of the test tubes which contained *P. felis*.

One of the test tubes which contained *Typhlopsylla musculi*.

The second lot of eight cultures gave only one positive result, i.e. one test tube which contained *P. canis*. The last eight cultures gave two positive results, and in both cases with *P. canis*. The positive results in these cases evidently depended on the fact that in the processes of sucking the tongue and mouth organs of the fleas retained a certain amount of infectious blood adhering to it.

The following experiment was undertaken with a view to proving how long the vitality of the plague microbe is maintained in the body of the flea under different conditions.

Experiment VII.

A number of each of the four species of fleas which had fed on a dying infected rat were subsequently starved. When they were dead, one test tube containing a corpse of each species was exposed to different conditions of moisture and temperature, and after a certain time tested for the presence of plague microbes. The conditions were the following:—

1. The first four test tubes containing each one flea of each of the kinds were put into the thermostat at the temperature of 28—30° C.
2. The second lot of four test tubes was subjected to the action of sunlight. The fleas treated in this manner were investigated daily.
3. The third lot of four test tubes was put into the cold cupboard with a temperature on an average of 3° C.
4. The fourth lot of four test tubes was placed at a temperature of from - 5 to - 15° C.
5. The fifth lot of test tubes was left at room temperature in a dark cupboard.
6. To the sixth lot of test tubes was added physiological salt solution and they were then placed in the same cupboard.
7. These fleas were kept between moist sheets of filter paper and pieces of linen.

The corpses of the fleas were investigated every three days to see whether plague bacilli could be obtained from them. The results are shown in Table V.

TABLE V.

Species of fleas	In the thermostat temp. 28°—30°	Room temp. 14°—16°	Physiological salt solution	Between damp sheets of paper and linen	Cold cupboard temp. +3°	Under the action of direct rays of sunlight	Freezing -5°—15° C.
<i>Pulex irritans</i>	3	9	21	24	18	3	15
<i>Pulex canis</i>	4	6	15	21	18	4	15
<i>Pulex felis</i>	3	6	21	18	21	3	12
<i>Typhlopsylla musculi</i>	2	9	18	21	15	3	18

The numbers indicate days of survival of the bacilli.

Cultures from fleas which had been kept between damp sheets of filter paper were tested for virulence on the second day from the beginning of the experiment. The rats which had infected these fleas had died from inoculation of a culture the virulence of which was $\frac{1}{10000000}$ c.c. From Table VI. it appears that the virulence had decreased to almost

TABLE VI.

Nos. of rats	Dose	Result	Time of death
1	$\frac{1}{1000000}$ c.c.	lived	—
2	„	lived	—
3	„	lived	—
4	$\frac{1}{750000}$ c.c.	lived	—
5	$\frac{1}{800000}$ c.c.	died	after 103 hours
6	$\frac{1}{500000}$ c.c.	died	after 97 hours
7	„	died	after 107 hours

one half ($\frac{1}{600000}$ c.c.). After passage of the culture three times through rats it regained its former virulence.

The following experiments show at what period of the illness of infected rats fleas absorbed plague microbes by sucking their blood.

Experiment VIII.

Four rats were infected with plague cultures of different strengths, and at intervals of 12 hours after infection three fleas of each of the four species were applied to them until they had fed sufficiently. These fleas were afterwards investigated microscopically and by culture. Blood from the infected rats was withdrawn at the same time and examined in the same manner. The feeding of the fleas was repeated at the above intervals until the death of the rats 72 to 108 hours after inoculation. The virulence of the culture inoculated varied somewhat, as will be seen from Table VII below, in which the results of the experiment are set out.

We see therefore that the plague microbe could be easily detected only in the fleas which contained the blood of the animal 12 to 26 hours before its death.

According to Yersin and Nuttall, flies die after feeding on a medium containing plague culture, or on organs of an infected corpse. They die sooner, the higher the temperature. The fleas, however, remained perfectly indifferent to the presence in their body of plague microbes, and if allowed to feed daily on infected rats they lived a long time both at room temperature and in the thermostat.

I next endeavoured to ascertain :

1. Whether plague microbes are present in the excretions of such fleas.
2. If so, during what length of time after application to an infected animal and subsequent feeding on healthy ones they could be found.
3. Whether the vitality and virulence of the plague microbe are preserved after its transit through the digestive canal of the flea.

Experiment IX.

One flea of each of the four species was placed in a small sterilised tube and applied to a moribund infected rat. They remained in this test tube for 24 hours, and after that were placed in others. Their faeces, which remained on the glass of the first test tubes in the form of black spots, were mixed with a drop of sterilised water at the end of

Plague Transmission by Insects

TABLE VII.

Length of time from inoculation of the rat with plague after which fleas were applied to them, and the fleas and the rats' blood investigated

Nos. of rats and the virulence of the culture used for the inoculation	Species of fleas	12 hours			24 hours			48 hours			60 hours			72 hours			84 hours			96 hours			108 hours			Time of death after
		Blood	Microscopical investigation	Culture	Blood	Microscopical investigation	Culture	Blood	Microscopical investigation	Culture	Blood	Microscopical investigation	Culture	Blood	Microscopical investigation	Culture	Blood	Microscopical investigation	Culture	Blood	Microscopical investigation	Culture	Blood	Microscopical investigation	Culture	
1	<i>Pulex</i>	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	after 98 hrs.
100000 c.c.	<i>irritans</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2	<i>Pulex</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	after 112 hrs.
100000 c.c.	<i>canis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
3	<i>Pulex</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	after 94 hrs.
100000 c.c.	<i>felis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
4	<i>Typhlo-</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	after 86 hrs.
100000 c.c.	<i>psylla</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
3	<i>musculi</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

+ plague bacilli present : - plague bacilli not found.

a platinum wire. The liquid obtained in this manner was spread on the surface of a slide which was afterwards dried, fixed and stained with methylene blue and eosin. Another portion of the liquid was poured into broth tubes and put for 24 hours in the thermostat.

After 24 hours the fleas were again placed in fresh test tubes and their faeces again investigated. It was only possible to do this during two days, as the unfed fleas died on the third day. The microscopical examination of the excreta gave the following results:

Amongst the amorphous masses which were faintly stained pink by eosin and small unstained balls with double outlines which reminded one of fat drops, oval bi-polar bacilli occurred which were stained by methylene blue. There were from three to ten bacilli in the field of vision; amongst these bacilli one could sometimes observe microbes of other forms, *e.g.* cocci of different sizes, but these were only present in insignificant numbers.

The broth cultures showed a growth after 24 hours. This growth did not show the characteristic appearances of plague cultures, and the microscopical investigation revealed that, in addition to oval bi-polar staining rods and chains which did not stain by Gram, cocci and also long thin non-motile rods were present.

Cultures of the excretions on agar revealed:

1. A considerable number of round-shaped colonies of grey colour which under the microscope looked like small seeds with double festooned edges, and which consisted of short bi-polar staining bacilli.

2. Smaller quantities of opaque colonies either white or yellow, and once green, consisting of bacilli and cocci of different sizes.

The first colonies when grown on agar and broth gave typical growths of plague. The second colonies I did not investigate, as they did not present any resemblance to plague colonies.

The faeces of infected fleas of all four species were investigated over 100 times and both on the first and second day invariably gave a positive result.

The following experiment was undertaken to determine after how many days the faeces of fleas which had been fed once on an infected animal, and subsequently on healthy rats, contain the plague microbe.

Experiment X.

One of each of the four species of fleas was applied after previous starvation to a dying infected rat. After 24 hours the fleas were placed in fresh test tubes and their faeces investigated in the same way as in

Experiment IX; after that they were fed on healthy rats and their excretions examined every 24 hours. This was continued for several days with the following results.

TABLE VIII.

Species of fleas	Nos. of fleas	Days after the beginning of the experiment										
		1	2	3	4	5	6	7	8	9	10	
<i>Pulex irritans</i>	1	+	+	+	+	+	-	-	-	-	-	
	2	+	+	+	+	-	-	-	-	-	-	
	3	+	+	+	+	+	+	-	-	-	-	
	4	+	+	+	+	-	-	-	-	-	-	
<i>Pulex canis</i>	1	+	+	-	-	-	-	-	-	-	-	
	2	+	+	+	+	+	-	-	-	-	-	
	3	+	+	+	+	+	-	-	-	-	-	
	4	+	+	+	+	-	-	-	-	-	-	
<i>Pulex felis</i>	1	+	+	+	-	-	-	-	-	-	-	
	2	+	+	+	-	-	-	-	-	-	-	
	3	+	+	+	+	+	-	-	-	-	-	
	4	+	+	+	+	-	-	-	-	-	-	
<i>Typhlopsylla musculi</i>	1	+	+	+	+	+	-	-	-	-	-	
	2	+	+	+	+	+	+	-	-	-	-	
	3	+	+	+	+	+	+	-	-	-	-	
	4	+	+	+	+	+	-	-	-	-	-	

+ plague bacilli present: - plague bacilli not found.

From the results set forth in Table VIII we see that the faeces of the infected fleas did not contain any plague microbes after five to six days, and the investigation of the fleas themselves proved that they did not contain any plague microbes after this period. We are led to suppose that the plague microbe is localised in the digestive canal without spreading to the insect's tissues and other organs.

It remained to be determined if the plague microbe preserves its virulence after being passed through the digestive organs of the flea. Agar cultures obtained from the faeces of *P. irritans* on the seventh day following their infection were tested, and it was seen that the strength of the culture had not decreased and corresponded in its virulence to the culture which had served to infect the rat. The experiments to determine how long and under what conditions this virulence is preserved in the faeces were made subsequently together with the experiments on infected bugs, and will be detailed later.

As may be seen from Table IX inoculation not only with cultures but with the faeces of the infected fleas themselves, caused the death of rats from plague.

Experiment XI.

The material used for inoculation in this experiment consisted of the faeces of an infected flea; they were mixed with physiological salt solution and subcutaneously injected into a rat. This was done every 24 hours, the flea being fed on healthy animals.

TABLE IX.

Species	Which day after infection the faeces were used for inoculation	Result on rat	Time of death
<i>Pulex irritans</i>	1	+	after 84 hours
	2	+	„ 95 „
	3	+	„ 103 „
	4	+	„ 97 „
	5	-	
	6	-	
<i>Pulex canis</i>	1	+	after 89 hours
	2	+	„ 103 „
	3	+	„ 97 „
	4	+	„ 101 „
	5	+	„ 108 „
	6	-	
<i>Typhlopsylla musculi</i>	1	+	after 105 hours
	2	+	„ 113 „
	3	+	„ 93 „
	4	-	
	5	-	
	6	-	

+ rat died : - rat lived.

These results show that a flea may excrete during three to five days a sufficient amount of plague microbes to kill a rat, but that inoculations with the faeces excreted later than five days gave a negative result. The post-mortem revealed in some cases a more, in others a less pronounced picture of plague, whilst an enormous number of plague microbes were to be observed in the blood and organs of the animal. On two occasions an abscess was observed at the point of inoculation. In the first case only yellow staphylococci could be obtained from the pus, and the rat remained alive; in the second case the pus contained plague microbes as well as yellow staphylococci, and the rat died of plague. The virulence of the culture used for infecting the rat on which the fleas were fed was $\frac{1}{1000000}$ c.c. of 24 hours' broth culture. When fleas were infected from rats which had been infected by a culture of a low virulence (M.L.D. $\frac{1}{100}$ to $\frac{1}{1000}$ c.c.) the excretions of these

fleas did not occasion infection and death from plague, although the excretions contained plague microbes.

Experiment XII.

If a certain number of fleas are infected by feeding on a dying rat and some of them crushed and at once examined microscopically, whilst the remaining fleas are allowed to live and their faeces examined microscopically daily, we find that the number of plague microbes is certainly larger in the latter case. On counting the number of microbes visible in the field of vision in preparations made from fleas crushed immediately after infection, and comparing the numbers with those in the blood of the rat on which they had fed, we find that the more microbes are contained in the blood, the more will be found in the fleas. But it is only rarely that one succeeds in seeing more than two to three plague microbes in the field of a specimen from a crushed flea. The reason is evidently that the blood sucked by the fleas is diluted by the juices of these insects and by the physiological salt solution employed in making the preparation. Fleas which have been once fed on an infected rat, and subsequently on a healthy one, show a considerable increase of plague microbes in their faeces after 24 hours. The number of these microbes in the field of vision is generally from eight to ten, and almost the same number was observed in the faeces on the second or third day; on the fourth day, however, the number decreased to two or three in the field of vision, on the fifth and sixth day the whole preparation hardly contained three to four microbes and not always even that, and after seven to eight days there were none to be found at all. The same results were obtained with all four species of fleas.

An additional observation was made with a view to proving that the plague microbe goes on increasing in the infected fleas.

Experiment XIII.

Pulex canis were infected as usual from a dying rat. Altogether 22 fleas were used, two in each of 11 test tubes. After feeding, two of them were at once crushed, and the remaining 10 test tubes containing 20 fleas were kept, five at room temperature and five in the thermostat at 28—30° C. The fleas were fed during the experiment on healthy animals. After every 24 hours two of each series were crushed and microscopically examined. Comparison of the number of microbes contained in the field of vision in the case of

fleas which were crushed at once, with the number contained in the preparations of fleas kept both at room temperature and in the thermostat, showed differences. In the former preparations from one to four plague microbes were found, in the preparations on the next day from fleas kept at room temperature there were four to seven, and from those kept in the thermostat from three to twelve. There was approximately the same number of microbes in the preparations made on the second and third day. On the fourth day, in both the fleas kept at room temperature and in the thermostat, the number suddenly decreased to one or two in the former case, and three to five in the latter. In some cases plague bacilli were found on the fifth and sixth day, but they entirely disappeared on the seventh to eighth day. It was clear therefore that the plague microbes increased in the digestive canal and the intestine of the flea, and that the increase is the more evident where the infected insects have been kept at a temperature favourable to the plague microbe, *i.e.* in the thermostat.

Experiments XIV to XVIII.

The question now was whether infected fleas played only a passive part in transferring infectious material, or whether they could actively spread plague through living alternately on diseased and healthy animals and inoculating the latter with plague by their bites. Only a minimal quantity of plague microbes can be communicated in this way, so that infection can only take place on condition that the plague microbe injected possesses a very high degree of virulence. This was proved by the experiment made with plague cultures of a low virulence, as in no cases did infection take place through fleas which had been infected by rats inoculated with a culture, the minimal lethal dose of which was $\frac{1}{100000}$ c.c., $\frac{1}{1000000}$ c.c., $\frac{1}{2000000}$ c.c., $\frac{1}{3000000}$ c.c., or $\frac{1}{4000000}$ c.c., notwithstanding that the fleas were found on bacteriological and microscopical examination to contain a number of living plague microbes. Five experiments were made. Ten rats were used for each experiment, and not one of the 50 rats died. In the nineteenth experiment the rat used for infecting the fleas was inoculated with a virulent culture, and a positive result was obtained for the first time.

Experiment XIX.

A rat was inoculated with plague culture the M.L.D. of which was $\frac{1}{5000000}$ c.c., the quantity injected was $\frac{1}{4000000}$ c.c. of broth culture. On

the fourth day its blood was examined during the death agony, and was seen to contain a considerable number of plague microbes; the rat was afterwards placed in a beaker, the inner surface of its left hind leg carefully shaved, and fleas of the four species, which had been previously starved for 30 hours, were applied. Altogether 30 fleas of each species, two or three in each test tube, were applied. After 12 hours they were allowed in the same manner to bite the shaven surface of the leg of healthy rats. Each rat was bitten by 10 fleas of one species, so that 12 rats altogether were used in the experiment. On the sixth day one of the three rats which were bitten by infected fleas of the species *P. canis* died; the blood and organs gave cultures typical of plague. The remaining rats did not show any signs of illness.

Experiment XX.

A rat was infected with a plague culture, of which the minimal lethal dose was $\frac{1}{800000}$ c.c. The experiment was conducted as above. Out of 12 rats three died; one on the fifth day, which had been infected by *Typhlopsylla musculi*, and two, on the fifth and sixth day respectively, which had been infected by *P. felis*. The blood and the organs of all three rats revealed a large number of plague microbes.

Experiment XXI.

The procedure was the same as in Experiments XIX and XX except that the rat was inoculated with a broth culture, the minimal lethal dose of which was $\frac{1}{1000000}$ c.c. Three out of 12 rats died. Two infected by *P. irritans* died on the fourth and fifth day, and the third infected by *P. canis* died on the fifth day.

Whenever positive results were obtained in the three last experiments there was at the situation of the bites a limited oedema or infiltration surrounded by an area in which the vessels were injected and, in some cases, small haemorrhages occurred.

Experiments were next made in which the fleas were simply placed in a vessel containing a rat so that they could themselves select the situation whereon to feed. These and all the further experiments were conducted exclusively with a culture with M. L. D. $\frac{1}{1000000}$ c.c.

Experiment XXII.

Ten fleas of one species were placed in a vessel containing a rat dying of plague. After six to eight hours, these fleas were removed and placed in a large cylindrical pot into which a healthy rat was afterwards placed. Generally, when the rat was taken out after half an hour there were no fleas left behind, as they had all settled on the rat. This experiment was repeated 10 times with each of the four kinds of fleas. Forty rats were used, out of which eight died.

In view of the well-known habit of fleas of leaving an animal as soon as it is dead, I made an experiment under conditions which as nearly as possible corresponded to those found in nature. I had a cage made which consisted of a zinc box, the sides of which had glass panes. Its length was $1\frac{1}{2}$ yards, the breadth and height 1 yard. This box was divided into two halves by a wire screen with openings of 3 mm. square; one of these halves, painted red, was reserved for infected animals, the other, painted white, for healthy ones. It was possible to entirely separate the two halves by pushing in a glass partition at 1 c.m. distance from the wire screen; it descended inside in the part where the healthy animal was kept. The bottom of the cage was made of perforated zinc, and the cage was placed upon a basin containing sublimate solution. The top of each half was separate and consisted of the very finest copper gauze, the openings of which hardly admitted the point of a needle; at all four corners were inserted movable boxes for containing solid and liquid food. The experiments were conducted in the following way.

The glass partition between the two halves was lowered, the infected rat put into one half and a healthy one into the other. A number of fleas were shaken from test tubes on to the infected rat when it was dying. After its death the glass partition was raised and the corpse allowed to remain for 24 hours in the cage; the healthy rat was removed on the next day. Not a single flea was discovered on the corpse after 24 hours; they had all settled on the healthy rat. This was especially marked in the experiments with *P. irritans*, *P. canis*, and *P. felis*.

The method followed in separating the fleas from the rat was the following: the rat was secured by means of a pair of pincers applied to its neck, and its hind legs were grasped by a hand wrapped in a towel soaked in sublimate. The animal was held over a glass jar, and carefully combed with a fine comb. The hands, rubber coat, and table on which this operation took place were soaked with sublimate. The jar was

afterwards covered with a funnel, on the top of which was placed a test tube; when the jar was reversed all the fleas fell down into the test tube where they were kept. Whenever it was necessary to take out a certain number of fleas, another smaller glass funnel was applied to the opening of the test tube, and the necessary number were decanted into another test tube.

Experiment XXIII.

This experiment with *Typhlopsylla musculi* was conducted in the cage described above. Fifteen fleas were applied to each of 10 infected rats. Out of the 10 healthy rats two died, one on the fifth and the other on the sixth day. All the fleas collected from these two rats contained plague microbes.

Experiment XXIV.

This was a similar experiment with fleas of the species of *P. canis*. Out of 10 healthy rats used for this experiment one died on the seventh day, and nine remained alive.

Experiment XXV.

A similar experiment was performed with *P. irritans* but gave a negative result. All 10 rats remained alive.

Experiment XXVI.

A similar experiment with *P. felis*; out of 10 rats one died on the sixth day.

These experiments establish that it is undoubtedly possible to convey plague from one rat to another by means of flea bites, though the percentage of illness and death in these cases is comparatively small.

The next experiments were performed in order to ascertain whether a wound made by non-infected fleas afforded a means of entrance, under favourable conditions, for any plague microbes coming into contact with it. Or whether those bites were only fatal when inflicted by infected fleas which forcibly introduced plague microbes under the skin at a certain depth below it.

Experiment XXVII.

From one to five fleas (not infected) were applied to the shaved surface of the hind leg of a number of rats. Pronounced traces of bites

were left on the skin, which disappeared on pressure and appeared again when the pressure was removed. The situations of the bites were smeared with

1. A 24 hours' broth culture of plague bacilli.
2. The same culture diluted 1—100.
3. The blood and organs of an infected animal.
4. Faeces of infected fleas.

A soft brush was used for spreading the different preparations so as to avoid any pressure and rubbing of the skin, and the material brought into contact with the place of the bites was allowed to dry on the skin. The rats were shaved the day before the experiment so as to allow the irritation of the skin to subside; the shaving itself was conducted very carefully and no animal which had received even the slightest cut was used. It must be mentioned that in this experiment only half the

TABLE X.

Material used for smearing the fleas' bites	No. of fleas which inflicted bites	Result in rat	Time of death
24 hours broth culture of plague bacilli	1	—	
	2	+	95 hours
	3	—	
	4	+	112 „
	5	+	101 „
24 hours broth culture diluted 1—100	1	—	
	2	—	
	3	—	
	4	—	
	5	+	93 hours
Blood of a rat which died from plague	1	—	
	2	+	87 hours
	3	+	94 „
	4	+	101 „
	5	+	77 „
Pulp from a kidney of an infected rat	1	—	
	2	—	
	3	+	79 hours
	4	—	
	5	+	78 „
Excretions of infected fleas	1	—	
	2	+	99 hours
	3	—	
	4	+	80 „
	5	—	

+ died: — lived.

animals were treated by shaving, in the others the hair was simply cut short: this proved quite immaterial to the fleas since they bit as readily in the one case as in the other.

The results obtained are set forth in Table X.

The results show that it is possible to infect animals in all the above mentioned ways. The fleas evidently inflicted sufficient injury to the skin to allow the plague microbes to penetrate it. In no case could the infection be brought about unless more than one flea had inflicted their bites. Positive results were obtained when the bites of two fleas were smeared with a plague culture of which the minimal lethal dose was $\frac{1}{1000000}$ c.c. and with the blood of a rat which was killed by inoculation with the same culture and also with the faeces of infected fleas. The same culture diluted 1—100 was only active when five fleas had previously inflicted their bites on the place to which the culture was applied.

Animals used for control which were shaved in exactly the same way and to whose skin the same materials were applied, but who had not been previously bitten by fleas, all remained unaffected. Moreover, if the infectious material was applied to rats 24 hours after the bites had taken place no infection was caused, however many fleas had been applied to the rat. It is apparent therefore that the healing of the skin which takes place in the first 24 hours is sufficient to protect the animal.

The following experiment was undertaken to determine how long after feeding on a septicaemic animal the flea is able to communicate infection. We have already shown that the plague microbe remains in the flea up to six days.

Experiment XXVIII.

Thirty of each of the four species of flea were applied to rats dying of plague in lots of 10 each. After 24 hours each lot of 10 fleas was fed on a healthy rat; this proceeding was continued for six days and each day fresh rats were used. The results obtained were as follows:

1. *Typhlopsylla musculi*. Out of three healthy rats bitten by fleas 24 hours after their own infection, one died. Out of three rats which were bitten by the same fleas 24 hours later, all remained alive. After another 24 hours one of the rats died. All the rats which were bitten after a longer interval remained alive.

2. *Pulex canis*. Three rats which were bitten by fleas 24 hours after infection remained alive. Out of three rats bitten by the same

fleas after another 24 hours, one died 68 hours after being bitten. Only negative results were obtained when the bites took place at a later period up to five days.

3. *Pulex felis*. Out of three rats bitten by fleas 24 hours after infection, one died after six days. After two days, one of the rats was infected and died. After three days and later no infection could be obtained.

4. *Pulex irritans*. No infection was obtained after 24 hours or 48 hours. The same fleas however infected one of three rats on the third day; later experiments gave negative results.

It follows therefore that fleas containing plague microbes can during the first three days infect healthy rats, but that after three days from infection the fleas were not capable of conveying the disease.

The following experiments were undertaken in order to determine what was the minimal number of infected fleas necessary to infect a healthy rat.

Experiment XXIX.

Several series of rats, 10 in each series, were treated with infected fleas of *P. canis* in the following way:

To each rat of the first series two were applied.

To each rat of the second series three were applied.

To each rat of the third series four were applied.

To each rat of the fourth series five were applied.

Rats belonging to the first three series all remained alive. From the rats belonging to the fourth series one died after 78 hours and one after 82 hours. This indicates that even when the plague microbe contained in infected fleas is highly virulent no infection is likely to take place when their number is less than five.

PART II. EXPERIMENTS WITH BUGS.

These experiments were conducted with guinea-pigs. The plague culture used came from Batum, but its virulence was enhanced by passing it several times through guinea-pigs. The bugs used were *Cimex lectularius* which is the usual domestic parasite. The strong irritation occasioned by its bite is caused by the action of its saliva which it injects into the wound. Though it is chiefly a human parasite it will nevertheless quite willingly bite mice, rats and guinea-pigs, but only if the skin of these animals is shaved on the place to which the bug is applied.

The bugs were contained in test tubes and were applied to the guinea-pigs by reversing the tube.

A bug never inflicts more than one bite and does not leave the place until it has filled itself with blood; its body under these conditions acquires an egg-shaped form. It is easy to judge what an enormous quantity of blood it can take in in comparison to its size by crushing it between two microscopical slides. One obtains enough liquid to smear the surface of both glasses, whilst the crushing of a starving bug leaves only small transparent spots.

The first thing to determine was whether bugs which had sucked the blood of an infected animal contained plague microbes. The experiments were conducted in exactly the same way as those with fleas.

Experiment XXX.

Bugs which had not been fed for 24 hours were applied to an infected guinea-pig dying of plague. Immediately after feeding some of them were crushed between two glasses and the contents of their bodies spread over the slides. These were fixed and stained for microscopical examination. Some of the bugs were also put into test tubes with broth, one into each, and crushed with a sterilised glass rod. The test tubes were afterwards placed in the thermostat.

On examining the film preparations one could observe amongst perfectly preserved blood corpuscles a considerable number of plague microbes which exhibited the usual bi-polar staining. The cultures obtained from crushed bugs were not pure but contained cocci in addition to plague bacilli. The latter were separated by plating and identified.

Two guinea-pigs were infected; one by inoculation with a pure culture obtained as above, another with the contents of five infected bugs. Both guinea-pigs died and the post-mortem examination of them gave a characteristic picture of plague. It follows therefore that bugs take in, together with the blood of the guinea-pigs, the plague microbe contained in the latter.

The following experiment was undertaken to determine how long the plague microbe existed in the bug and whether it lost its virulence under those conditions. As will be seen the result depends upon the length of the starvation to which the bugs were subjected previous to feeding on an infected animal. The longer the previous starvation, the longer was the time during which the plague microbe could be recovered,

and *vice versa*. In the case of bugs which had not been starved before their infection or only starved for a short time, the plague microbe was only recovered for a short period.

Experiment XXXI.

Ten series of bugs, 50 in each series, were starved:—

- | | |
|--------------------------------|---------------------------------|
| (1) For 24 hours. | (2) For 7 days. |
| (3) For 2 weeks. | (4) For 3 weeks. |
| (5) For 1 month. | (6) For 2 months. |
| (7) For $2\frac{1}{2}$ months. | (8) For 3 months. |
| (9) For 4 months. | (10) For $4\frac{1}{2}$ months. |

At the end of the period of starvation the bugs of each series were fed upon septicaemic guinea-pigs inoculated with a plague culture of which the M.L.D. was $\frac{1}{1000000}$ c.c. of a 24 hours' broth culture; they were afterwards examined in the following manner. Five bugs were taken daily during 10 days. One of them was crushed between two slides and prepared for microscopical examination, another was crushed on a watch glass by a sterilised glass rod and its contents aspirated into a pipette, inoculated into broth and incubated for 24 hours. The last three bugs were also crushed on a watch glass and their contents, with the addition of a small quantity of physiological salt solution, were used for inoculating guinea-pigs.

These experiments were continued for five months. Bugs were chosen of the same size and each was placed separately into a small test tube. They were applied to the guinea-pigs in these test tubes after which they were placed in a cupboard at 16—18° C. The results will be seen in Table XI.

The plague microbe could be recovered after from one to two days if the bug had been starved from one to seven days before feeding upon an infected guinea-pig. If it had been kept starving from fourteen to thirty days the plague bacillus could be detected up to the third day. After thirty days or ninety days starvation the microbe was found to be still present on the sixth day, and after a period of starvation of four to four and a half months it could still be found on the eighth day¹.

¹ In Nuttall's experiments (*Centralbl. f. Bakt.* xxii. (1897), p. 92) *Cimex lectularius* which had starved for three months were fed on septicaemic plague mice. The bugs were kept at 20° C. and their contents injected at intervals into mice. Virulent bacilli survived for 72 hours (but not for 120 hours) in the bugs. Smear preparations of the gut contents of the bugs made 24 hours after feeding on infected blood revealed an almost pure culture

The guinea-pigs presented at the post-mortem the characteristic picture of bubonic plague with haemorrhagic infiltration at the place of inoculation, bubo and enlargement of the cervical and axillary glands. The bubo was the size of a pea or small bean, of a deep red colour in the guinea-pigs which had died early, and of a yellowish tinge in those which had died late. The kidneys were enlarged with a great number of grey points under the capsule, and the liver was enlarged and dark or yellowish. In the blood and organs a large number of plague microbes were present.

The microscopical investigation of crushed infected bugs revealed that the plague microbes preserved their morphological characteristics during all the time that they were found in the bodies of the bugs.

The preparations from crushed bugs of the first two series were, up to the second day after their infection from guinea-pigs, identical with the preparations obtained from the blood of these guinea-pigs; in the midst of a great mass of well preserved blood corpuscles could be seen an enormous quantity of plague microbes in almost pure culture. After two days no blood corpuscles could be detected in the preparations of the infected bugs, and the number of plague microbes decreased suddenly and they were entirely absent in the preparations from bugs crushed three days or longer after infection. The same picture was presented by the preparations obtained from bugs which had been starved from 14 to 30 days before being fed on an infected guinea-pig (series 3, 4, 5), with the only difference that no plague microbes could be detected in them after four days, instead of three days, from the time of the infection. In the preparations from the bugs starved from two to four and a half months (series 6 to 10) before infection, the blood corpuscles of the guinea-pig were preserved much longer; they could be found on the third and even the fourth day after feeding on the infected guinea-pigs. The number of plague microbes found was highest at that time, and their number gradually decreased and disappeared entirely on the seventh day in series 6, 7 and 8, whilst in series 9 and 10 they continued to be found till the eighth day.

In these preparations from crushed infected bugs one could often find cells which contained plague microbes; these cells were the same size as the leucocytes of the guinea-pig and were like them, but for the fact that they contained a large number of vacuoles. Sometimes these

of *B. pestis*, but after 48 hours they appeared to be distinctly fewer in number and more saprophytes were present. The longer survival found by Dr Verbitski is doubtless due to the lower temperature at which he kept his bugs.—Ed.

TABLE XI.

No. of the series	Period of pre-vivisection before application to the infected guinea-pigs	Number of days after infection when the examination of bugs by microscope, culture and inoculation, was undertaken																			
		1st day		2nd day		3rd day		4th day		5th day		6th day		7th day		8th day		9th day		10th day	
		Microscopical investigation	Culture	Microscopical investigation	Culture	Microscopical investigation	Culture	Microscopical investigation	Culture	Microscopical investigation	Culture	Microscopical investigation	Culture	Microscopical investigation	Culture	Microscopical investigation	Culture	Microscopical investigation	Culture	Microscopical investigation	Culture
		hrs.	hrs.	hrs.	hrs.	hrs.	hrs.	hrs.	hrs.	hrs.	hrs.	hrs.	hrs.	hrs.	hrs.	hrs.	hrs.	hrs.	hrs.	hrs.	hrs.
1	24 hrs.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	7 days	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	14 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	21 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	30 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	60 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	75 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	90 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	120 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	125 "	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ plague bacilli found, or guinea-pig inoculated with contents of bug died, with time of death after inoculation.
 - plague bacilli not found, or guinea-pig remained healthy.

cells contained red blood corpuscles. At first I considered these cells to be the leucocytes of the blood of the guinea-pig. I found afterwards however that Tictin¹ has described such cells in preparations made from bugs which had fed on human and monkey blood. Tictin considers them to be derived from the bugs, an opinion with which I am inclined to agree. This view is supported by the fact that these cells could be observed in preparations from bugs which had been starved for a considerable time, and also in preparations of infected bugs which had been crushed at a period after infection when red blood corpuscles of the guinea-pig could no longer be detected.

Experiment XXXII.

This experiment was made to determine whether a sojourn in the alimentary canal of bugs affected the virulence of the organisms. Cultures were taken which had been obtained in Experiment XXXI from crushed infected bugs (series 9 and 10), one of which bugs had been crushed on the fifth, the others on the eighth day after infection. These cultures were tested on guinea-pigs. Three guinea-pigs were inoculated with $\frac{1}{1000000}$ c.c. of the culture from the bug of series 9 and three from that of series 10. After inoculation with the first culture all three guinea-pigs died after 61, 58 and 66 hours respectively; with the second culture two died, one after 57 and the other after 63 hours; the third remained alive. This proves that even a lengthy stay inside the body of the bug did not rob the plague microbes of their original virulence.

Experiment XXXIII.

Three guinea-pigs were inoculated with a plague culture. Every six hours afterwards three bugs were allowed to feed on them. The bugs were immediately examined microscopically and by culture, as described in Experiment XXXI. At the same time the blood of the guinea-pig was examined microscopically.

The results are set out in Table XII from which it will be seen that as soon as bacilli were discoverable in the blood they were detected in the crushed bugs.

¹ *Materials for the Study of Relapsing Fever*, Dissertation, 1898.

TABLE XII.

Number of hours after inoculation of guinea-pigs with plague culture, when their blood was examined, and bugs were applied to them, which in their turn were examined

Numbers of guinea pigs	6 hours												12 hours												18 hours												24 hours												30 hours												36 hours												42 hours												48 hours												54 hours												60 hours												66 hours												72 hours												Time of death of the guinea pigs after inocula- tion																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																										
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+ plague bacilli found: - plague bacilli not found.

Experiment XXXIV.

Forty-two bugs which had been starved for two months were applied to an infected guinea-pig at a period of its illness when its blood on microscopical examination revealed the presence of only two or three bacilli in each field of vision. Two of these bugs were at once crushed and microscopical examination of their contents showed one or two plague bacilli in each field of vision. Of the remaining 40 bugs, 20 were placed in the thermostat at 28—30° C. and 20 in the cupboard at 14—16° C. Four of each lot were crushed each day. After 24 hours the microscopical investigation showed that the number of plague microbes had increased, there being now five to six in each field of vision in the case of those kept at 14—16° C., and in those kept in the thermostat 10 to 15. The same quantity of microbes was found in the preparations of bugs examined two days after infection. After three days the number of bacilli began to diminish, preparations from four bugs which had been kept at room temperature showing two to three in each field, and those kept in the thermostat five to six. Still fewer could be found in preparations made on the fourth day, and on the fifth day they could rarely be found.

Plague bacilli evidently multiply in the intestines of the bug. In those cases where the bugs had been weakened by starvation and in which the blood was only slowly digested, the plague microbes were found to persist for a longer time (Experiment XXXI).

Experiment XXXV.

Twelve bugs which had been starved for a month were fed on an infected guinea-pig, two were crushed at once and plague microbes were found under the microscope. Of the remaining bugs five were put into the thermostat, and five into a cupboard at 14—16° C. All were alive after two months so that the feeding on infected blood does not appear harmful to the insects themselves.

The following experiments were made to discover whether the faeces of the infected bugs contained plague microbes. Infected bugs were kept in small sterilised test tubes. Their faeces accumulated on the walls of these tubes in the form of black specks, sometimes 1 mm. in diameter. They were semi-liquid immediately after feeding on the animal, but if no further feeding occurred became less in quantity and ultimately ceased.

Experiment XXXVI.

Four bugs previously starved for more than a month were applied to a dying guinea-pig, each in a separate test tube; on the following day they were each placed into a fresh test tube and their faeces investigated in the following way. The faeces of one bug were dissolved by a few drops of sterilised distilled water on a platinum wire. Samples were examined microscopically and also inoculated into broth. The excretions of the remaining three bugs were dissolved in 0.5 c.c. of sterilised distilled water and injected subcutaneously into three white mice and two guinea-pigs, 0.1 c.c. into each. The microscopical examination revealed five to seven plague microbes in each field of vision. Other bacilli were also present in these preparations and some large cocci. A pure culture of plague was obtained with difficulty by plating; all the animals inoculated with this culture died and at autopsy presented the ordinary picture of plague. Pure cultures of *Bacillus pestis* were obtained from the blood and organs.

The excretions of the same bugs 48 hours after their infection were investigated in the same manner both microscopically and by culture. A smaller number of plague microbes was observed. Two white mice and two guinea-pigs were inoculated, which all died from plague.

In the excretions on the third day the number of plague microbes was very small, and out of two mice and two guinea-pigs used for inoculation, one guinea-pig died after 77 hours. On the fourth and fifth days the faeces were free from plague bacilli.

This experiment proves that the excretions of bugs, which had been kept starving for a month previously to being fed on infected animals, contained virulent plague microbes for three days after feeding.

Experiment XXXVII.

Bugs were fed upon a guinea-pig which had been inoculated with a plague culture, of which the M.L.D. was $\frac{1}{1000000}$ c.c. This animal subsequently succumbed to plague. The culture obtained from the faeces of the bugs on the third day after their infection was tested for virulence. To this end four guinea-pigs were inoculated subcutaneously with $\frac{1}{1000000}$ c.c. of a 24 hours' growth in broth. Two of them died after 70 and 74 hours respectively, and one remained alive. The plague microbe therefore retains its virulence after passing through the intestine of the bug, as noted by Nuttall. In no case did the microbes

in preparations from crushed bugs present any morphological change or alteration in staining.

The next series of experiments had as their object the determination of how far infected bugs may be capable of producing infection through their bites.

Experiment XXXVIII.

Ten bugs, before they had absorbed as much blood as they wanted from an infected animal, were removed and applied immediately to a healthy guinea-pig.

In five experiments in which the original guinea-pigs had been inoculated with a culture of low virulence not one of the fresh animals died nor fell ill, though the bugs were found to contain plenty of plague microbes on microscopical examination.

The first positive result was obtained when the guinea-pigs used for infecting the bugs were themselves infected by a culture of which the minimal lethal dose corresponded to $\frac{1}{800000}$ c.c. Using a guinea-pig infected with a culture of this virulence, ten bugs were applied to each of fifteen healthy guinea-pigs. After 63 hours one of these guinea-pigs died, after 67 hours another. A pure culture of plague was obtained from their blood and organs. Thirteen guinea-pigs remained alive.

This experiment was repeated when the virulence of the culture had reached $\frac{1}{1000000}$ c.c. Of 26 guinea-pigs used for this experiment, four died in from 56 to 73 hours after the bugs had been allowed to bite them.

This comparatively small percentage of positive results increased suddenly when the infected bugs were applied to the ear instead of the hind leg of the guinea-pigs¹. The surface of the ear was carefully shaved, it was taken hold of with two fingers, and a test tube which contained the infected bugs was reversed with the opening downwards on to the upper shaved surface of the ear. In this experiment 10 infected bugs were again applied to each guinea-pig. Altogether 25 guinea-pigs were used, of which seven died from plague. There was no local reaction on the spot where the infection had entered the guinea-pig's body when the bites were inflicted on the ear.

Further experiments were undertaken to determine:—

Firstly, what was the smallest number of infected bugs which could bring about infection.

¹ Nuttall (1897, p. 92) made four experiments with mice with negative results: two to ten bugs were applied at the root of the tail.—Ed.

Secondly, how long bugs retained the power of infecting through their bites, and

Thirdly, if it was possible to infect several animals by means of the same infected bugs.

Experiment XXXIX.

One infected bug was, immediately after having fed on an infected guinea-pig, applied to each of the first series of healthy guinea-pigs. Two bugs were applied to each of the next series, etc., so as to determine the minimal number of bugs capable of infecting a healthy animal with plague. Each series consisted of five guinea-pigs. The bugs were applied to the skin of the left ear.

All the guinea-pigs belonging to the first and second series remained alive. One guinea-pig from the third series died after 64 hours. Four infected bugs were applied to each of the following two series. Three guinea-pigs died out of the 10, one after 53 hours, the second after 64 hours, and the third after 79 hours. Post-mortem examination showed in all three guinea-pigs, and in the guinea-pig belonging to the third series, a characteristic picture of plague. There was an insignificant subcutaneous infiltration on the place of the bite in the last named animal; the others did not show any traces of skin injury to indicate the place through which the infection had entered the body.

The smallest number of infected bugs which can transmit infection and cause the death from plague of a healthy animal appears therefore to be three.

Experiment XL.

This experiment was undertaken in order to ascertain how long infected bugs are capable of transmitting infection through their bites. Ten series of bugs which had been allowed to starve for about four months were applied to guinea-pigs dying from plague. There were 25 bugs in each series. Five of the infected bugs of the first series were applied to each of five healthy guinea pigs three days after infection. Of the second series, again five bugs were applied to each of five guinea-pigs four days after infection; the bugs of the third series were treated in the same way after five days, of the fourth series after six days, of the sixth series after seven days, etc.

Two guinea-pigs died from the bites of the bugs belonging to the first series, one after 49 hours, the other after 50 hours. One guinea-pig died from the bites of the bugs belonging to the second series

after 51 hours. The bites of the bugs belonging to the remaining series did not give any positive results, which allows us to conclude that the bug will not transmit infection after five days from the time when it had bitten an infected animal. All the bugs in this experiment were applied to the ear of the guinea-pigs.

The following experiment was undertaken with a view to determine whether several guinea-pigs could be infected through the bites of the same bugs.

Experiment XLI.

A guinea-pig was infected with a culture of which the M.L.D. was $\frac{1}{200000000}$ c.c. The guinea-pigs used in the previous experiment had been infected with the same culture. Thirty bugs which had previously been starved for two months were fed on the guinea-pig. On each of the following days, five bugs were applied to the ear of each of six healthy guinea-pigs, taking fresh animals each day. When fed on the infected animals the bugs were not allowed to take as much food as they would have liked to do, as I found that if they were allowed to do so they would not bite at all, or only unwillingly, on the next day. Two guinea-pigs out of six which were bitten by the bugs immediately after infection died after 50 hours; the bites of the same bugs 24 hours later killed one guinea-pig after 58 hours. All three guinea-pigs undoubtedly died of plague. The condition of the ear in all three was perfectly normal; the anatomical examination showed an ordinary picture of plague and the blood and organs and bubo were full of plague microbes. Further applications of the same bugs to guinea-pigs on the following days gave no results.

This indicates that the same infected bugs cannot convey plague infection more than twice in the course of the first and second day; and that they were not able to infect on the third or fourth day, notwithstanding that they still contained plague microbes on the fifth day.

Under natural conditions the bug causes irritation of the skin by crawling, and is often crushed on the place where it inflicts its bite, thereby spreading its contents on both the skin and the clothing. A bug is much more easily crushed than a flea, especially when it is full of blood. Favourable conditions would therefore be created for plague microbes to penetrate any cracks in the skin. The following experiment was undertaken to investigate this point.

Experiment XLII.

The inner surface of the hind legs of six guinea-pigs was slightly scratched three times with a very fine needle and the contents of crushed infected bugs were spread over the place. The bugs used for the first three guinea-pigs were crushed 24 hours after their infection. Those used for the second series of three guinea-pigs had been infected four days previously. The bugs had been kept starving for about two months before applying them to the infected guinea-pig. All the six guinea-pigs died of plague 47 to 69 hours after being subjected to this treatment.

Experiment XLIII.

One infected bug was applied to the shaved ear of each of ten guinea-pigs; five guinea-pigs received bugs which had been infected 24 hours previously, the remaining five were bitten by bugs four days after infection. Instead of using ordinary test tubes in this experiment I had small glass tubes open at both ends and stopped with cotton wool. The infected bug contained in one of these tubes was applied to the ear of the guinea-pig. When the bug became swollen with blood the cotton wool was removed from the further end of the tube and the bug crushed on the spot with a glass rod.

Seven of the 10 guinea-pigs died; all the five belonging to the first series, and two belonging to the second. A small amount of subcutaneous oedema and haemorrhagic infiltration were found in two of them at the situation of the bite, the remaining five had no local signs; buboes were present in the neck of the size of a small bean; the internal organs showed a typical appearance and, as well as the blood, contained enormous quantities of plague microbes.

This shows that the comparatively small percentage of plague infections transmitted through bug bites is considerably increased when the bugs are crushed on the skin of the animal bitten.

In the experiments with fleas it was shown that the injury to the skin made by their bites is sufficient to allow plague microbes to gain entrance. The corresponding experiment was undertaken with bugs.

Experiment XLIV.

One uninfected bug was applied to the ear of each of 30 guinea-pigs. Different kinds of infectious plague material were immediately applied to the spots. In the first five pigs they were smeared with

a 24 hours' broth culture; in the second five with the blood of an infected guinea-pig; in the third five with the faeces of infected bugs; in the fourth five with a broth culture diluted 1—100, and in the remaining ten partly with the blood of a guinea-pig which had died of plague and partly with a 24 hours' broth culture. In the case of the last ten, however, the applications were not made at once but 24 hours after the bites had been inflicted.

Two guinea-pigs which had been treated with the broth culture died after 49 and 57 hours. Two guinea-pigs treated with the blood of a dead infected animal and the excretions of infected bugs died, the former after 54 hours, the latter after 77 hours.

All the guinea-pigs in which the infective material was applied 24 hours after infliction of the bites remained alive. All dead guinea-pigs presented the characteristic picture of bubonic plague. Control animals were treated in exactly the same manner without having been previously bitten by bugs; they all remained alive.

This proves that the injury to the skin inflicted by the bites of bugs is sufficient to allow infectious material to penetrate into the organism, but that after 24 hours the little wound is entirely healed and no infection can penetrate through it.

The clothing of people who live in dirty unhygienic surroundings is generally covered with spots from crushed bugs and their faeces. It is possible that the plague microbes contained in these spots may be capable of transmitting infection, and it was therefore important to establish how long the plague microbes will preserve their vitality in these spots under different circumstances.

I began by trying the effect of drying the spots and the microbes contained in them, but this treatment seems to destroy them. Experiments with the faeces of infected fleas, and with fleas crushed on different fabrics, were undertaken at the same time.

Experiment XLV.

Bugs were applied to guinea-pigs dying from plague. When they were fully fed, a proportion of them were crushed with a glass rod inside a test tube and their contents were used to soil sterilised strips of linen, cotton, silk and woollen fabrics, half a centimetre wide; they were also spread over sterilised strips of the same size of filter and writing paper, glass slides and small pieces of pine wood. The remainder of the infected bugs was kept in sterilised glass basins, the bottom of which

was covered respectively with filter and ordinary paper, linen, wool, cotton and silk fabrics. After three days the paper and materials were covered with the faeces of the insects in the form of black spots of different sizes.

The strips of materials and paper soaked with the contents of the infected bugs were cut into smaller pieces of about half a centimetre square and placed in Petri dishes. The slides and wood smeared with the same material were also placed in Petri dishes. The paper and various fabrics covered with faeces were cut into smaller pieces so as to have two or three spots on each piece, and these also were put into Petri dishes.

Pulex canis were after infection treated slightly differently; a few drops of sterilised water were introduced into the test tube and the fleas allowed to drown in it. After this they were placed between two glasses which had been previously covered on the surfaces facing each other with filter and writing paper, linen, silk and other fabrics, and crushed. The spots obtained from the crushed fleas were cut out and placed in Petri dishes. To obtain the faeces of fleas for investigation the inner surface of a test tube was lined with sterilised paper and various other fabrics. The fleas contained in these test tubes left their faeces on the fabrics and paper. These were treated in the same manner as those of the bugs.

In order to determine on the one hand the action of drying accompanied by the action of diffused light, Petri dishes containing the same material were placed in the thermostat at 28—30° C. and in a dark and a light cupboard at room temperature (14—16° C.). The dishes were not covered with lids but with discs of filter paper.

The investigations were conducted in the following manner: the pieces of material, after submission to the various treatments, were put into test tubes containing broth and incubated. If growth was present after 24 hours, a microscopical examination was made. If this revealed the undoubted presence of plague bacilli no further investigation was made. Where there was the slightest doubt plating was resorted to. If no growth of plague microbes could be detected after 24 hours, the tubes were left in the thermostat for a few more days after which the cultures obtained were examined both under the microscope and by plating. If no plague microbes could be detected on the ninth or tenth day, the result of the experiment was considered to be negative. The longer the previous drying process, the later the growth of the plague microbes occurred, appearing in some cases only on the fourth or fifth day.

The saprophytes, which were mostly cocci, grew in broth earlier than the plague bacilli but they did not conceal or prevent the growth of the latter. When separated in pure culture and inoculated into broth at the same time as the plague microbe, they grew there side by side, each species retaining its vitality. Even in the case when plague microbes were put into broth in which there already was a growth of these cocci, the bacilli succeeded in growing. The results obtained in this experiment are illustrated by the following Table.

TABLE XIII

Material used	Fabric	Thermostat 30° C.	Room temperature	
			Dark cupboard	Diffused light
Crushed infected bugs	Linen	4	45	42
	Cotton material	3	20	19
	Silk „	3	16	18
	Wool „	4	18	18
	Filter paper	2	18	15
	Writing „	2	7	5
	Glass	2	6	6
	Wood	3	11	8
Faeces of infected bugs	Linen	3	18	14
	Cotton material	2	16	16
	Silk „	3	10	10
	Wool „	3	12	12
	Filter paper	2	15	15
	Writing „	2	5	3
	Glass	1	6	5
Crushed infected fleas	Linen	3	22	19
	Cotton material	3	18	17
	Silk „	2	17	13
	Wool „	2	18	18
	Filter paper	3	16	16
	Writing „	2	5	4
	Glass	1	5	5
Faeces of infected fleas	Linen	3	12	10
	Cotton material	2	6	6
	Silk „	2	7	7
	Wool „	3	7	4
	Filter paper	2	6	5
	Writing „	1	6	4
	Glass	1	6	5

The numbers indicate days of survival of the bacilli.

The fact that the microbes contained in the spots from crushed fleas died sooner than those contained in the spots from crushed bugs can be explained by the difference in the depth of the layer in these

spots; the fabrics treated with crushed bugs were soaked, whilst the crushing of the fleas only left traces on the surface. The additional moisture in the first case provided more favourable conditions for the life of the bacillus. Even in spots from crushed fleas and their faeces, plague microbes remained alive during a considerable time, longer on linen, silk, cotton and wool fabrics and filter paper (16 to 22 days) than on glass and writing paper (5 days).

It is clear therefore that spots from crushed infected fleas, bugs and their excretions can at an ordinary temperature contain plague microbes during 18 to 45 days, and it is easy to realise the danger presented by the underclothing of patients ill with plague with regard to infection, if plague microbes can preserve their vitality on drying for a month, *i.e.* under the most unfavourable circumstances. It is natural to suppose that in moist surroundings they will live still longer.

Experiment XLVI.

In this experiment the same materials as were used in Experiment XLV were placed in glass dishes which, in their turn were placed open into large Koch dishes, at the bottom of which was sterilised water up to the height of 1 centimetre to prevent drying. This was replaced as it evaporated. Some of the dishes were covered with lids and placed in the thermostat, others were kept in the dark cupboard, and others exposed to diffused light at room temperature, others again in the cold cupboard at 4° to 5° C. The examination of materials treated in this way took place every three days in the same way as in the last experiment.

TABLE XIV.

Material used	Fabric	Thermostat 28°—30° C.	Room temperature 14°—18° C.		Cold cupboard 4°—5° C.
			Dark cupboard	Diffused light	
Crushed infected bugs	Linen	30	141	130	168
	Cotton material	30	135	120	162
	Silk ,,	28	130	130	162
	Wool ,,	28	123	118	129
	Filter paper	18	118	118	120
	Writing ,,	18	99	90	120
	Glass	18	99	93	120
Faeces of infected bugs	Linen	18	33	30	48
	Cotton material	12	33	33	36
	Silk ,,	12	21	18	39
	Wool ,,	9	21	21	48
	Filter paper	12	18	18	18
	Writing ,,	12	18	12	12
	Glass ,,	9	18	12	18

TABLE XIV (*cont.*).

Material used	Fabric	Thermostat 28°—30° C.	Room temperature 14°—18° C.		Cold cupboard 4°—5° C.
			Dark cupboard	Diffused light	
Crushed infected fleas	Linen	42	121	121	159
	Cotton material	42	121	105	159
	Silk „	28	105	105	115
	Wool „	39	118	121	159
	Filter paper	21	99	99	121
	Writing „	21	90	90	105
	Glass	21	93	81	99
Faeces of infected fleas	Linen	15	24	24	33
	Cotton material	15	18	12	39
	Silk „	9	18	9	33
	Wool „	12	21	12	27
	Filter paper	12	18	9	18
	Writing „	9	18	12	18

The numbers indicate days of survival of the bacilli.

The action of direct sunlight and of freezing on the microbes contained in these stains was next investigated.

Experiment XLVII.

The investigation of the action of direct sunlight was undertaken in the beginning of June. Material treated in the same way as in the two previous experiments was exposed to the direct rays of the sun in Petri dishes from 11 a.m. to 5 p.m. Every half hour from the beginning of the experiment, bits of the fabrics, paper, glass, etc. were taken out and put into broth. In the cases where the plague microbes had not died after one day's exposure they were exposed again on the following days, weather permitting. The thermometer was placed in the dishes which contained the material under investigation. I did not undertake any precautions to prevent the preparations getting heated by the sun, therefore my preparations were dried by the sun whilst exposed to its rays.

TABLE XV.

Fabric	Temperature	Hours of survival in direct sunlight			
		Spots from crushed bugs	Spots from their faeces	Spots from crushed fleas	Spots from their faeces
		Hours	Hours	Hours	Hours
Linen	38—46° C.	12	6½	9½	5
Cotton material	„	8½	5	6	5
Wool „	„	9	4½	6	3½
Silk „	36—41° C.	6½	5	5½	4½
Filter paper	„	2½	1½	2	1
Writing „	40° C.	2½	1	1½	1½
Glass	„	2½	1	1½	1

Experiment XLVIII.

To investigate the influence of freezing on plague microbes on the same materials, they were placed in Petri dishes wrapped in thick black paper and put outside the window in the winter. All the materials were examined daily. The temperature to which the dishes were exposed was from -5° to -18° C.

TABLE XVI.

Fabric	Temperature	Days of survival			
		Spots from crushed bugs Days	Spots from their faeces Days	Spots from crushed fleas Days	Spots from their faeces Days
Linen	-5 to -18° C.	16	9	12	9
Cotton material	„	14	9	12	7
Wool „	„	14	10	9	9
Silk „	„	11	9	10	7
Filter paper	„	9	8	6	7
Writing „	„	8	7	6	5
Glass	„	6	4	5	5

We see therefore that plague microbes in the faeces of these insects remain alive from four to 16 days at a temperature of from -5° to -18° C.

It was next ascertained whether the virulence of the plague bacilli was preserved unaltered under the influence of these surroundings.

Pure cultures of plague were prepared from the following sources :

(1) Crushed infected bugs on bits of linen :—

- (a) Dried during 35 days at room temperature.
- (b) Kept during 130 days in damp surroundings at a temperature of 4° — 5° C.
- (c) Exposed during 8 days to direct sunlight.
- (d) Frozen during 10 days at a temperature of -5° to -18° C.

(2) From crushed infected fleas on linen after :—

- (a) Drying during 15 days at room temperature.
- (b) After being kept 120 days in damp surroundings.
- (c) Exposed for 6 hours to direct sunlight.
- (d) Frozen during 7 days at -5° to -18° C.

(3) From the faeces of bugs which had been allowed to dry on linen at room temperature during 10 days.

(4) From the faeces of fleas on the same fabric which were kept in damp surroundings at a temperature of 4° — 5° C. for 15 days.

All the above cultures were tested for their virulence by inoculating guinea-pigs (cultures obtained from bugs) or rats (cultures obtained from fleas).

The guinea-pigs which had served originally to infect the bugs had been inoculated with a culture of which the M.L.D. was $\frac{1}{2000000}$ c.c. to $\frac{1}{4000000}$ c.c.; the rats which served to infect the fleas had been inoculated with a culture, the minimal lethal dose of which was $\frac{1}{1000000}$ c.c. The cultures obtained from the various materials above mentioned were tested for virulence by inoculating guinea-pigs and rats with a gradually increasing dose. The results were as follows:—

In the case of cultures from linen soiled with crushed bugs and fleas or the faeces of these insects which had been exposed for six to seven hours to the action of direct sunlight, the minimum fatal dose for guinea-pigs was $\frac{1}{100000}$ c.c. (death on the fourth to eighth day), and for rats $\frac{1}{20000}$ c.c. (death on the fifth to ninth day). Cultures from the same materials upon linen which had been exposed for seven to 10 days to a temperature of -5° to -18° C. killed guinea-pigs in the dose of $\frac{1}{500000}$ c.c., and rats in the dose of $\frac{1}{250000}$ c.c. The virulence of the organism which had dried gradually during 15 to 35 days at room temperature was much better preserved. These cultures killed guinea-pigs in a dose of $\frac{1}{1000000}$ c.c. (three to nine days), and rats in a dose of $\frac{1}{250000}$ c.c. (on the sixth day). The virulence decreased least in spots on linen which was kept damp in the cold cupboard (3° to 5° C.). Cultures obtained from these killed guinea-pigs at $\frac{1}{1500000}$ c.c., and rats at $\frac{1}{500000}$ c.c. The guinea-pigs died after three to five days, the rats after four to six days.

From the results one must conclude that clothing and bed clothes which are covered with material from infected insects, obtained either by crushing them or from their faeces, can serve during a long time as a source of infection.

Disinfectants. Experimenting with the ordinary chemical disinfectants I found that corrosive sublimate 0.1 per cent., phenol 5 per cent. or formalin 1 per cent. kill plague bacilli in the above fabrics, in squashed bugs or fleas, or in the blood of infected animals in ten or fifteen minutes. They also kill fleas at once, but do not destroy plague bacilli within the fleas for three to four hours. Even 0.2 per cent. sublimate takes 2 to $2\frac{1}{2}$ hours to penetrate the flea. Formalin vapour is a poor insecticide especially with bugs.

SUMMARY OF RESULTS.

(1) All fleas and bugs which have sucked the blood of animals dying from plague¹ contain plague microbes.

(2) Fleas and bugs which have sucked the blood of animals which are suffering from plague only contain plague microbes when the bites have been inflicted from 12 to 26 hours before the death of the animals, that is, during that period of their illness when their blood contains plague bacilli.

(3) The vitality and virulence of the plague microbes are preserved in these insects.

(4) Plague bacilli may be found in fleas from four to six days after they have sucked the blood of an animal dying with plague. In bugs, not previously starved or starved only for a short time (one to seven days), the plague microbes disappear on the third day; in those that have been starved for 4 to 4½ months, after eight or nine days.

(5) The numbers of plague microbes in the infected fleas and bugs increase during the first few days.

(6) The faeces of infected fleas and bugs contain virulent plague microbes as long as they persist in the alimentary canal of these insects.

(7) Animals could not be infected by the bites of fleas and bugs which had been infected by animals whose own infection had been occasioned by a culture of small virulence, notwithstanding the fact that the insects may be found to contain abundant plague microbes.

(8) Fleas and bugs that have fed upon animals which have been infected by cultures of high virulence convey infection by means of bites, and the more certainly so the more virulent the culture with which the first animal was inoculated.

(9) The local inflammatory reaction in animals which have died from plague occasioned by the bites of infected insects is either very slight or absent. In the latter case it is only by the situation of the primary bubo that one can approximately identify the area through which the plague infection entered the organism.

(10) Infected fleas communicate the disease to healthy animals for three days after infection. Infected bugs have the power of doing so for five days.

¹ That is, if a septicaemia is present. Dr Verbitski does not appear to have had the experience that rats may die of plague without developing a septicaemia sufficiently extensive to infect the flea: this not infrequently occurs especially in cool climates.—Ed.

(11) It was not found possible for more than two animals to be infected by the bites of the same bugs.

(12) The crushing of infected bugs *in situ* during the process of biting, occasioned in the majority of cases the infection of the healthy animal with plague.

(13) The injury to the skin occasioned by the bite of bugs or fleas offers a channel through which plague microbes can easily enter the body and occasion death from plague.

(14) Crushed infected bugs and fleas and their faeces, like other plague material, can infect through the small punctures of the skin caused by the bites of bugs and fleas, but only for a short time after the infliction of these bites.

(15) In the case of linen and other fabrics soiled by crushing infected fleas and bugs on them, or by the faeces of these insects, the plague microbes can under favourable conditions remain alive and virulent during more than five months.

(16) Chemical disinfectants do not in the ordinary course of application kill plague microbes in infected fleas and bugs.

(17) The rat flea *Typhlopsylla musculi* does not bite human beings.

(18) Human fleas do bite rats.

(19) Fleas found on dogs and cats bite both human beings and rats.

(20) Human fleas and fleas found on cats and dogs can live on rats as casual parasites, and therefore can under certain conditions play a part in the transmission of plague from rats to human beings, and *vice versa*.

EPIDEMIOLOGICAL SIGNIFICANCE OF THE RESULTS OBTAINED.

The results described above show that the part played by insects in the epidemiology of plague must be considerable. Taking into consideration on the one hand how difficult it is to bring about the infection of a rat through the digestive canal, and on the other hand the ease with which the plague microbe penetrates through the most insignificant scratches on the skin, the transmission of infection from one animal to another through the agency of rat fleas, which leave a dead and infected rat to go and live on other healthy rats, to whom they in turn communicate plague infection, affords a perfectly satisfactory explanation of plague epidemics amongst rats.

This supposition is proved by the facts

(1) that all fleas found on rats which die of plague contain plague microbes¹;

(2) that the bites of such fleas can occasion infection with plague in healthy animals when these fleas are artificially transferred on to fresh animals, and when this transference of infected fleas from a dead animal to a healthy one takes place voluntarily.

Concerning the communication of plague from rats to human beings, numerous observations have shown that cases of plague have been occasioned by people coming into contact with infected rats, for instance by touching them with their hands, and that it is more dangerous to touch warm corpses than those that had already become cold. Though rat fleas do not bite human beings² still a certain number of the fleas of domestic animals may be present on rats, and under certain circumstances also human fleas which readily bite both men and rats.

During plague epidemics in China and India the buboes observed have been chiefly situated in the groin, which proves plainly that the infection must have chiefly entered the body through the legs and feet of individuals. In India where the native quarters are teeming with fleas it is, according to Blackmore, just the feet which suffer more than any other part of the body from bites.

Infection from man to man, except in the "pneumonic" variety, may no doubt be explained by the transmission of the plague infection through insects. The way in which bugs convey plague to man is probably the same as that suggested by Tictin in the case of relapsing fever. A bug which has not succeeded in filling itself with infected blood transfers itself to a healthy individual and forthwith bites again, thereby introducing any plague bacilli adherent to its proboscis. Or owing to the irritation it produces it may be crushed by the individual in his efforts to capture it, and the infective contents rubbed into the slight puncture occasioned by the bite.

Bugs which have sucked their full complement of blood do not as a rule bite again for a considerable interval, but if felt crawling upon the skin may be crushed in the same way. Should they contain plague bacilli these may be inoculated through any slight abrasion existing in the neighbourhood.

¹ Dr Verjbitski presumably means in his experience. This was not by any means the case with the observations of the Commission.—Ed.

² This is of course not true for the Indian rat flea (*P. cheopis*): see these reports *Journal of Hygiene*, vol. VII. (1897), p. 472.—Ed.

Besides this direct part which infected insects can play in the spread of plague, the belongings and especially the linen of plague patients soiled by the faeces of these insects or their crushed bodies can serve as a possible source of plague infection during a long time. This may explain how clothes of people who have died of plague may transmit infection even months after their possessor's death.

XXVII. REPORT ON EXPERIMENTS UNDERTAKEN TO
DISCOVER WHETHER THE COMMON DOMESTIC
ANIMALS OF INDIA ARE AFFECTED BY PLAGUE¹.

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the Government of India.)

IN a report on plague in Hongkong submitted to the Secretary of State for the Colonies in December 1902, Professor Simpson² says (p. 8) "The experiments undertaken demonstrate that pigs, calves, buffaloes, sheep, hens, ducks, geese, turkeys and pigeons are, in addition to rats, susceptible to plague, and particularly so when fed with plague material." He points out that these experiments have a twofold importance for the Health Officer, viz.

(a) that the ordinary farmyard animals will have in future to be watched, and regarded as possible sources of plague, and

(b) that the eating of contaminated food may—in spite of the contrary opinion of the Indian Plague Commission—be a common way of acquiring plague, and that therefore inspection of food stuffs assumes a new and important aspect.

The opinion held in India has always been that these animals were immune to plague, and it seemed therefore necessary to reinvestigate the matter with regard to local conditions here, and measures were

¹ The original of this paper was a report submitted to the Government of India in 1904.

² *Report on the causes and continuance of Plague in Hongkong and suggestions as to Remedial Measures* by W. J. Simpson, M.D., F.R.C.P., London, Waterlow and Sons, Ltd., Printers, London Wall, 1903.

taken by Mr Haffkine to accomplish this. Accordingly, in 1904, two pigs (one boar and one sow) and two ducks (one duck) were fed with the heart, spleen, liver, lungs and rats which had died of plague.

Owing to Mr Haffkine's departure at the end of April nothing further was done till the 12th of May, when feedings were resumed and extended by us as detailed below.

The following animals were used:—

4 pigs,	2 calves,	4 fowls,
4 turkeys,	4 geese,	4 ducks.

The methods described by Professor Simpson were followed exactly as possible, the only difference being in the fact that the feed given to the animals were in rats' bodies, not in human bodies as in Hongkong experiments.

Rats were injected with plague germs derived from the blood and grown in broth. During the course of the experiment 64 were used for feeding purposes. Of these 64 died within two days of infection, 30 within three days, and only three lived longer than three days. There can be no doubt therefore that the plague germs used were virulent. After death, specimens were made from the blood for microscopic examination, and cultures on agar were taken for the stalactite test. If the microscope revealed the presence of plague-like bacilli the rat was used for feeding one of the other animals in the following manner.

The head, bladder, and intestines were removed, and the rest of the body and organs cut into small pieces. These were mixed with the usual feed of bran which the animals were used to eat. The bran was boiled and cooled down before the pieces were mixed with it. None of the animals refused to take this mixture. Many of them ate greedily, picking out the rat flesh with their mouths. Latterly, the minced rat flesh was mixed with sterile sand and given to the animals without mixture with bran.

To be certain that the rats used had really died of plague, cultures in broth were made from the agar tubes reserved for

therefore that the germs used were those of virulent plague capable of killing rats, in from 2 to 3 days. The germs were derived from human sources.

The following is the result of the experiments.

Pigs.

Pigs Nos. 1 and 2, full grown male and female, were fed seven times each as above described. On each occasion one rat was given to each. The feeding took place on the following dates: 18th March, 12th, 13th, 16th, 19th and 21st May, and 8th June, 1904. The pigs at no time showed signs of illness, and are still alive and well.

Pig No. 3, full grown female, was on 18th June, 1904, scarified on the left shoulder. Into the scarifications was rubbed the juice of the liver of a rat which died of plague derived from a human source. The animal did not take its food properly for two days, but then quickly recovered.

The site of scarification was inflamed for a couple of days but after this the wounds rapidly healed.

This animal is also alive and well at date of writing, three months later.

Pig No. 4, full grown male, was on 18th June, 1904, injected subcutaneously with 2 c.c. of an emulsion of the organs of a rat which had died of plague. The emulsion was made with sterile broth and the germs were derived from a human source. This animal did not eat much for a day or two, and an abscess developed at the site of inoculation. This was opened on 30 June 1904, and had healed on 8 July 1904.

The pig is still alive and well.

Calves.

Two calves about 9 months old were fed 7 times each as above on the following dates:—24th, 26th and 28th May, and 1st, 3rd, 4th, and 11th June 1904.

Neither animal showed any signs of illness, and both are alive and well at the time of writing 3 months afterwards.

when it succumbed to an attack of chicken cholera induced in it by subcutaneous injection.

Fowl No. 2, a full-grown cock bird was fed 5 times as above, on 10th, 11th, 12th, 13th and 16th June 1904. The bird showed no signs of sickness at any time, and is still alive and well.

Fowl No. 3, a full-grown hen, was scarified on the neck and under the wing on the 16th June 1904. Into these scarifications was rubbed a piece of liver of a rat which had died of plague. No reaction was produced, and the animal is still alive and well.

Fowl No. 4, a full-grown cock, was injected subcutaneously on 18th June, 1904, with emulsion of the internal organs of a rat which had died of plague. The bird suffered no inconvenience but died on the 4th July, 16 days afterwards, of some undiscovered cause. On post-mortem examination no trace of disease could be found.

Smears from liver and heart blood were free from microbes, and cultures from liver and heart blood remained sterile.

Turkeys.

Turkey No. 1, a full-grown cock bird was fed 8 times as above described, on the 20th, 21st, 22nd, 24th, 26th and 27th May, and 4th and 9th June 1904. This bird suffered no inconvenience, and remained well up to 22nd July, when it was fed with the organs of turkey No. 2, which had died of chicken cholera. It gradually became much emaciated and died on the 19th August. Post-mortem examination revealed nothing as to cause of death. Smears from liver and heart's blood were free from microbes and cultures from the same remained sterile.

Turkey No. 2, a full-grown hen bird was fed as above described 8 times, on the following dates:—22nd, 26th, 27th May and 1st, 2nd, 5th, 6th, and 9th June 1904. This bird remained unaffected and well up to 20th July when it received a subcutaneous injection of a culture of chicken cholera, from the effects of which it died two days later.

Turkey No. 3, a full-grown hen was scarified on the neck and under the wing on 18th June 1904. A piece of liver from a rat which had died of plague was rubbed into the scarifications. The bird suffered no inconvenience, and is alive and well to this day.

Turkey No. 4, a full-grown cock was injected subcutaneously on 18th June 1904, with an emulsion made from the internal organs of a rat which had died of plague. The bird suffered no inconvenience and was well up to 20th August 1904, when it died during the rainy season

from some unknown cause. Smears of liver and heart blood were free from microbes and cultures from these remained sterile.

Geese.

Goose No. 1, a full-grown gander, was fed 7 times as above described on the following dates:—23rd, 26th and 27th May and 1st, 2nd, 5th and 10th June 1904.

This bird showed no signs of illness, and it was well up to 21st July 1904, when it was fed on organs of goose No. 2 which had died of chicken cholera, from the effects of which it died on the 29th July.

Goose No. 2, a full-grown goose was fed 7 times as above described on the following dates:—27th and 29th May and 1st, 4th, 5th, 6th, and 11th June 1904. This bird showed no signs of illness up to 20th July, when it received an injection of chicken cholera from the effects of which it died next day.

Goose No. 3, a full-grown goose was scarified on the neck and a piece of liver from a rat which had died of plague was rubbed into the place on the 18th June 1904. The bird suffered no inconvenience, and is still alive and well.

Goose No. 4, a full-grown gander, received subcutaneously on 18th June 1904 an emulsion of the internal organs of a rat which had died of plague. The bird suffered no inconvenience and is still alive and well.

Ducks.

Duck No. 1, a full-grown drake, was fed 7 times as above described on the following dates: 18th March, 16th, 19th, 21st, 22nd and 24th May and 8th June 1904. This bird showed no signs of illness at any time during this somewhat protracted period of feeding but died of some unknown cause on 24th June, 16 days after the last feeding. Post-mortem examination revealed no cause of death. Smears from liver and blood showed no micro-organisms, and the cultures from the same sources remained sterile.

Duck No. 2, a full-grown female bird, was fed 7 times as above described on the following dates: 18th March, 19th, 22nd, 26th, 27th and 28th May and 8th June 1904. The bird suffered no inconvenience, and is alive and well up to date.

Duck No. 3, a full-grown female bird, was scarified on the neck and the place was well rubbed on 16 June 1904 with a piece of liver of a rat

which had died of plague. The bird suffered no inconvenience, and is alive and well up to date.

Duck No. 4, a full-grown drake, was, on 18th June, injected subcutaneously with an emulsion of the internal organs of a rat which died of plague. The bird suffered no inconvenience, and is still alive and well.

The above experiments were, in every case, entirely negative, and it was not considered necessary further to extend the series, as the results previously obtained in this and other laboratories in India have been identical.

When Mr Haffkine in 1897 endeavoured to elaborate a curative serum for plague on the lines laid down by Roux and Yersin, the animals he used were horses, cattle, sheep, and goats; and he expressly says in his evidence before the Indian Plague Commission¹ that he used no animals that were susceptible to plague.

The German Plague Commission also experimented with various domestic animals in Bombay; 2 doves, 2 cocks and 2 geese were injected with virulent plague cultures, but showed no reaction. Two young pigs similarly injected showed no reaction. Two young pigs fed on plague rats also showed no sign of illness. Two sheep, one scarified, and one injected, showed high fever for four days, and formation of local abscess. The pus contained plague bacilli but both animals recovered. Two goats showed similar results, but the pus was sterile. Four cows, 2 scarified and 2 injected, showed a similar reaction but all recovered. They remark in conclusion, "In judging of these experiments it must not be forgotten that the infection was more intense than is met with in natural circumstances²."

Lieut. Walton, I.M.S., when working for the Indian Plague Commission, tried to infect pigeons with plague by hypodermic injection and failed³.

Beyond the confines of India, we find that numerous species of birds have been experimented with. Thus London⁴ made extensive experiments in Russia, with the common birds of that country. The birds used were pigeons, cocks and hens, ducks, crossbills, yellow-hammers, linnets and canaries. An emulsion of culture on agar of 24 hours' growth

¹ *Report of Indian Plague Commission*, Vol. I. p. 14, Q. 133.

² *Centralblatt f. Bak.* 1897.

³ *Indian Plague Commission Report*, Vol. III. p. 337, Q. 26,315.

⁴ *Les Oiseaux sont-ils sensible à la peste bubonique?* par M. E. S. London, *Archives des Sciences Biologiques*, publiées par l'Institut Impérial de Médecine Expérimentale à St Pétersbourg, Tome VI., No. 1, p. 67. St Pétersbourg, 1897.

was used in the majority of cases. Two pigeons received a culture made on pigeons' serum which had been deprived of bactericidal properties by heat. One hen was inoculated under the skin with a fragment of the spleen of a white mouse dead of plague. Not only were normal birds used, but also those whose vitality might be supposed to be lowered by such proceedings as the following: starvation, removal of the feathers from the thorax, abdomen and legs, and chilling of the body by immersion in cold water. White mice inoculated at the same time as the birds invariably died within 2 days. The conclusion arrived at by the author is that birds, either in a natural condition or when subjected to such treatment as above described, are not susceptible to plague.

Since the experiments above detailed were completed, the laboratory has received from Mr Ernest Hill, Health Officer for the Colony, a "Report on the Plague in Natal 1902-1903¹" in Part II of which Mr H. Watkins-Pitchford, F.R.C.V.S., Government Bacteriologist, details trials he had made to test the domestic animals of South Africa in accordance with Professor Simpson's plan. He says, "Repeated endeavours have been made to induce a fatal form of the disease by the ingestion of infected materials and by the inoculation of virulent cultures and blood, as well as by close contact with an animal in a highly infectious condition." Of 20 hens used, none became ill in any way; of 10 pigs, both English and Kaffir, none died and only "one showed a transient illness due to manipulation, not to plague"; of two calves neither was affected.

It appears quite certain therefore that the farmyard animals of India and Natal are not liable to contract plague either by ingestion, scarification, or subcutaneous injection, and need not be regarded with suspicion during plague epidemics. This is quite in accordance with Indian experience up to the present.

Buffaloes were not experimented with owing to difficulty in procuring a specimen, but as these animals swarm in Bombay City and are valuable property, it is certain that plague amongst them would long ere this have been reported.

It will be interesting in conclusion to analyse Professor Simpson's experiments with the aid of the details furnished in his report, to discover if any other explanation is possible than the supposition, that the animals of China belong to a breed so different as to succumb in

¹ *Report on the plague in Natal, 1902-1903*, by Ernest Hill, M.R.C.S., L.R.C.P., D.P.H. (Cantab.), Health Officer for the Colony. Printed by Cassel & Co., Ltd., London, Paris, New York and Melbourne, 1904.

numbers to a disease against which similar animals in India and Natal are immune.

Professor Simpson used 17 pigs in all. Of these two were not inoculated with plague material because they spontaneously developed some disease and died. On post-mortem examination "plague bacilli were found in the spleen, blood and glands" (p. 72) of one of them (pig No. 16). This animal was confined "in a pen adjoining those in which pigs 8, 9 and 10 were located." The second pig No. 17 "was not in the same pen as the foregoing and died on the 14th day." No plague bacilli were found in the pig nor were any inoculation experiments performed with its tissues. Material from pig 16 however was given by the mouth to six rats, four of which "died with plague bacilli in their blood" (p. 98). Two days before the death of this pig, Nos. 8 and 9 had died in an adjoining pen, and the organs of No. 8 when given to 6 rats caused the death of 5, in 4 of which plague bacilli were found. There was ample time for infection to spread from these pigs to No. 16, as they were confined in adjacent pens for seven days before they died.

It seems almost certain then that these three pigs Nos. 8, 16 and 17 were suffering from the same highly infectious disease, which was communicable to rats by feeding, and caused by a bacillus indistinguishable from the plague microbe by the methods used by Professor Simpson. These methods are not clearly described in the report, all that is stated being the following (p. 50): "Cultures from the internal organs of some of the animals experimented on were made and examined, particularly from calf No. 6, sheep No. 4, hen No. 7, pigs Nos. 7 and 13, monkey No. 4 and a goose and a pigeon. The cultures correspond to characteristics of ordinary plague bacilli, while those of pig No. 13, hen No. 7, and sheep No. 4 inoculated into guinea-pigs caused their death in two or three days with plague bacilli in their blood."

The remaining 15 pigs, presumably kept in pens in the same place as the above, were fed or inoculated subcutaneously or by scarification, with emulsions of various organs of animals which had died of plague. One animal received in addition an injection of an agar culture of plague. To prove that these animals died of plague, microscopical examination seems to have been relied on, save in the case of pigs No. 7 and No. 13 noted above. In these cases cultures were made, but on what media is not stated, and no mention is made of any differentiating tests having been applied. Without such tests, mistakes might arise from the coincidence of acute diseases due to organisms of the "chicken-cholera" or "hog-cholera" groups.

In support of this idea is the case of pig 13 which, it is acknowledged, may have died of "swine typhoid." It had a high temperature at the time it was fed with plague material, and died on the fifth day after feeding. "Plague bacilli were however found in blood, spleen, glands, and kidneys. Whether this animal died of plague or swine typhoid is difficult to say" (p. 70). Six rats were fed on the internal organs of this pig, and four of them "died with plague bacilli in blood" (p. 98). Now this was eminently a case for differential cultivation, as there was doubt as to the cause of death; whether this was done or not it is impossible to say from the details given in the report.

In this laboratory we have frequently been called upon for diagnosis of diseases causing death among the animals in the Victoria Gardens of Bombay, and in many cases have found bacilli indistinguishable by microscopical inspection from plague bacilli. So like are they in form and bi-polar staining reaction, that officers who have daily worked here with plague bacilli for years have been deceived when shown such preparations. In most cases these have proved to be the microbe of chicken cholera, easily communicable by feeding or injection to birds, as already noted above (*vide* Fowl No. 1; Turkeys Nos. 1 and 2; Geese Nos. 1 and 2). This microbe was found pathogenic for guinea pigs, but not for rats, calves or pigs. In calves a local reaction with abscess formation was observed, and swelling of the nearest chain of glands; in pigs, local abscess formation and sloughing, but nothing further.

It is interesting to note incidentally, that one of the workers in the laboratory, while making a *post-mortem* examination of a swan which had died of chicken cholera, inoculated himself accidentally by running a splinter of bone into his finger, and in consequence suffered from a sharp attack of fever and formation of a local abscess. Recovery was complete in a week. Or had this worker contracted plague at the same time, which he might quite well have done, his case would have been on all fours with that of the Chinese butcher who died of plague after having scratched his hand at a *post-mortem* on a pig. The growth of the chicken cholera microbe on agar is also very like that of plague, and it is only by further cultivation and inoculation experiments that they can be distinguished with certainty.

The suspicion therefore arises that the pigs used by Professor Simpson in Hongkong, may have been suffering from "hog-cholera," or as he calls it "swine-typhoid," which appears to be very common in China, or possibly from the *pasteurellose du porc* (Sweineseuche of the Germans). Thus Staff-Surgeon Wilm of the Imperial German Navy

quoted at p. 49 of Professor Simpson's report, gives an account of the *post-mortem* appearances found in a pig which had eaten the spleen of a man that had died of plague, which corresponds almost word for word with the classical descriptions of the lesions of hog-cholera. Professor Simpson further quotes the following from the same observer:—"In the beginning of August 1896, on board of two steam-ships carrying pigs to Hongkong from the island of Hainan and from Pakhoi respectively, a large number of pigs died. A great many also died after they had been landed at Hongkong. *Post-mortem* examination of the bodies of these animals showed the same morbid appearances as in those killed by giving them plague-infected organs to eat, the appearances being most marked in the intestinal tract." Wilm found the bacillus pathogenic to "mice, rats, guinea pigs and rabbits when injected subcutaneously," but these also are just the laboratory animals susceptible to the "hog-cholera" bacillus.

At the time Wilm made these experiments and observations, the methods of differentiating these various organisms were undeveloped, and the stalactite test for plague had not been discovered by Mr Haffkine. Wilm's cultural tests would not now be regarded as conclusive and his inoculation ones as we have seen are open to another interpretation, viz. that the pigs died of hog-cholera and that the bacillus associated with that disease was mistaken for the plague microbe.

That this might easily happen is evident when we find the "hog-cholera bacillus" thus described by Theobald Smith¹:—"They are short rods with end rounded, not producing spores....They usually present a more deeply stained periphery and give the impression of a bacillus completely filled out by a feebly stained spore"; and again (p. 10) "In cover-glass preparations from the tissues of inoculated animals, the central portion of the rod is frequently only feebly stained." These are descriptions that might quite well be given of the plague bacillus.

It is instructive to place in parallel columns the description of symptoms and *post-mortem* appearances observed by Professor Simpson in his pigs, and those of the text-books describing hog-cholera.

That acute swine diseases are very common in Southern China appears from Appendix F of Professor Simpson's report. Thus, in answer to the question "Has there been any illness prevalent among pigs or cattle preceding the plague outbreak?" we find the following

¹ *Additional Investigations concerning Infectious Swine Diseases* by Theobald Smith, Ph.B., M.D., and Veranus A. Moore, B.S., M.D., published by authority of the Secretary of Agriculture, Washington Government Printing Office, 1894, p. 22.

Professor Simpson's account of the symptoms and *post-mortem* appearances in the pigs.

1. *Duration* of disease was from the 4th to 36th day after feeding : average about 3 weeks.
2. "Remained apparently quite well with no diarrhoea until a day or two before death when they appeared to be somewhat feeble on their legs and inclined to stagger" (p. 51).
"Suffered from diarrhoea" (p. 52).
"The pigs seemed to be feeble on hind legs" (p. 51).
"Unsteady gait before death" (p. 52).
3. "The eyes became congested and discharged white mucus in which plague bacilli were found" (p. 52).
"Both had congestion of eyes" (p. 52).
4. *Post-mortem* appearances : "Congestion of the organs, congestion and haemorrhagic condition of most of the lymphatic glands, specially of the throat and neck" (p. 53).
"Glands of the body were congested, but varied very much in their degree of congestion and haemorrhagic state" (p. 53).
"The lymphatic glands being congested and haemorrhagic" (p. 54).
"Patchy inflammation and haemorrhages into mucous membrane of the large intestines" (p. 51).
"The large intestines were congested and haemorrhagic" (p. 51).
"The large intestines inflamed in patches" (p. 52).
"Showed inflamed large intestines" (p. 52).
"*Post-mortem* showed lungs to be pneumonic in patches" (p. 52).
"Showed...lungs with pneumonic patches" (p. 52).
5. "Plague bacilli were present in the blood, spleen, glands, kidneys, bladder and intestinal contents" (p. 51).
Similar notes occur as to distribution of bacilli in almost all the pigs.

Text-book description of hog-cholera¹.

1. *Duration* from 1 or 2 days to 2 or 3 weeks.
2. "The animals often die suddenly without having appeared particularly ill," or "after seeming ill but a few hours" (p. 413).
"The bowels may be normal or constipated at the beginning of the attack; but later, there is generally a liquid and foetid diarrhoea" (p. 413).
"The animal becomes weak...and walks with a weak tottering gait."
3. "The eyes are congested and watery, the secretion drying and glueing the lids together" (pp. 413, 414).
4. "*Post-mortem* appearances: Extravasations of blood are common in the lymphatic glands" (p. 414).

"Extravasations of blood are common...beneath the serous membrane of the thorax and abdomen and particularly along the intestines."
"The contents of the intestines are sometimes covered with clotted blood" (p. 414).
"In hog-cholera the first effect of the disease is believed to be upon the intestines, with secondary invasion of the lungs" (p. 415).
"Occasional broncho-pneumonic changes in the lungs" (p. 415).
5. "The specific bacillus² of hog-cholera was secured by Smith from the spleens of more than 500 hogs. It occurs in all the organs and has been cultivated from the urine" (p. 415).

¹ *Text-book upon the Pathogenic Bacteria*, by Joseph McFarland, M.D., 2nd edition, Henry Kimpton, London, 1898.

² Recent observations of Dorset Bolton and McBride (*U.S. Dept. of Agriculture Bull.* 72, 1905, p. 101) have shown that the primary cause of hog-cholera will pass through a filter, and throw grave doubt on the aetiological position of the "hog-cholera bacillus."

statements: Dr A. Rennie of Canton reports "Epidemics amongst pigs are frequent in south China, but are more probably swine-fever (typhoid) than plague." Dr A. Lyall of Kwangtung says "Epidemics of pig disease are common of which they (Chinese) recognise two kinds: (a) with diarrhoea, (b) a disease in which a skin becomes 'red.'" Dr J. P. Maxwell of Changpoo says "As to pigs, what the Chinese call 'pig plague' is probably swine-fever and is certainly not usually bubonic plague." Many others to whom these questions were addressed also remark on epidemics among pigs, cattle and buffaloes in China, so that the elimination of these disturbing factors by a system of quarantine and strict segregation was important. No evidence that this was done is to be found in Professor Simpson's report, and it is possible that some at least of the results obtained may have been due to the prevalence of some sort of swine-plague among his animals.

XXVIII. ADDITIONAL OBSERVATIONS ON THE SEPTIC- AEMIA IN HUMAN PLAGUE WITH AN ACCOUNT OF EXPERIMENTS ON THE INFECTIVITY OF THE EXCRETA.

IN a previous paper (This *Journal*, vol. VI. p. 524) we have given an account of the quantitative estimation of plague bacilli in the blood of 16 septicaemic cases. It remains now to supplement this number with a further series and to add some observations on the infectivity of the urine and faeces.

PART I. FURTHER OBSERVATIONS ON THE SEPTICAEMIA IN HUMAN PLAGUE.

On account of the additional work involved in examining the excreta, the technique of the blood examinations previously described was discontinued and the following simpler method was used. By means of a sterilised syringe 2 c.c. of blood was removed from a suitable vein at the bend of the elbow, and 0.1 c.c. of blood, carefully measured by the graduation on the stem of the syringe, was transferred to each of two sloped dry agar tubes, the blood being spread as uniformly as possible over the surface of the tubes by shaking them. A specimen was then prepared for microscopical examination. The agar tubes after incubation were examined and subcultures of any colonies that had appeared were sown into flasks for the stalactite test. Animal tests (cutaneous or subcutaneous inoculation of guinea-pigs) were resorted to if considered necessary.

The details of the present series have been arranged in Tables I and II.

The data relating to the fatal cases, whose blood was examined on at least two occasions, are collected in Table I in such a form that the course of the septicaemia is easily seen.

Usually, as is to be expected, the septicaemia goes on increasing till death. Case 9, however, affords an illustration of a type to which we referred in our former paper, namely, a septicaemia that diminishes as the disease progresses. Case 10 is a remarkable one. Bacilli were

TABLE I. *Fatal cases in which B. pestis was recovered from the blood.*

Serial no.	Hospital no.	Quantitative estimation of septicaemia			Microscopical examination of blood	Time reckoned from hour of death	Time reckoned from date of attack	Estimated duration of illness
1	845	2000 per c.c.	Negative	67 hours	4 days	6 days
		Numerous isolated colonies—						
		0.1 c.c.	„	43	5	
		Uncountable	„	19	6	
2	817	2000—3000 per c.c.	„	58	4	6
		2000—3000 „	„	34	5	
		Fine layer—0.1 c.c.	„	10	6	
3	826	50 per c.c.	„	102	3	7
		50—60 per c.c.	„	78	4	
		A fine layer—0.1 c.c.	„	54	5	
4	778	50 per c.c.	„	47	3	4
		2000 „	„	23	4	
5	851	Sterile—0.1 c.c.	„	96	7	11
		Good growth—0.1 c.c.	„	72	8	
		Numerous—0.1 c.c.	„	48	9	
6	887	20 per c.c.	„	49	6	8
		200 „	„	25	7	
		Layer of just isolated colonies						
		—0.1 c.c.	„	1½	8	
7	823	Many isolated colonies—						
		0.1 c.c.	„	15½	4	5
		Almost a layer—0.1 c.c.	„	1½	5	
8	886	60 per c.c.	„	49	6	8
		300—400 per c.c.	„	25	7	
9	765	A few colonies—0.1 c.c.			„	25	5	7
		Sterile—0.1 c.c.	„	1½	6	
10	842	Very few—0.1 c.c.			„	17 days	8	26
		A few „	„	16	9	
		Fair number „	„	15	10	
		Sterile „	„	14	11	
		Few „	„	12	13	
11	854	Few „			„	5½	4	9
		Very few „	„	4½	5	

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TABLE II. *Giving results of examination of samples of blood
(urine and faeces not examined).*

Serial no.	Hospital no.	Quantitative estimation of septicaemia	Microscopical examination of blood	Time reckoned from hour of death	Time reckoned from date of attack	Estimated dura- tion of illness
Group I. Very numerous colonies.						
1	823	Almost a layer—0·1 c.c.	„	1½ hours	5 days	5 days
2	887	Layer of just isolated colonies—0·1 c.c. ...	„	1½	8	8
3	780	Very many isolated colo- nies—0·1 c.c. ...	„	8	Unknown	Unknown
4	860	A layer	„	11½	3 days	3 days
5	873	Very numerous isolated colonies—0·1 c.c. ...	„	23½	4	5
6	851	Good growth	„	72	8	11
Group II. Numerous colonies.						
7	814	Many isolated colonies 0·1 c.c.	„	2 hours	5 days	5 days
8	808	„ „ „ „ „	„	17½	2	3
9	864	„ „ „ „ „	„	25	3	4
10	823	„ „ „ „ „	„	25½	4	5
11	845	„ „ „ „ „	„	43	5	6
Group III. Fairly numerous colonies.						
12	862	1000—2000 per c.c. ...	„	Just before death	Unknown	Unknown
13	818	400—500 „ ...	„	27 hours	4 days	5 days
14	856	500—600 „ ...	„	29	Unknown	Unknown
15	868	1000—2000 „ ...	„	30	5 days	6 days
16	817	2000—3000 „ ...	„	34	5	6
17	817	2000—3000 „ ...	„	58	4	6
18	845	About 2000 „ ...	„	67	4	6
19	826	500—600 „ ...	„	78	4	7
20	842	Fair number—0·1 c.c. ...	„	15 days	11	26
21	866	500—600 per c.c. ...	„	Recovery	2	Recovery
Group IV. A few colonies.						
22	869	10 per c.c. ...	„	Recovery	2 days	Recovery
23	867	10 „ ...	„	Just before death	20	20 days
24	886	300—400 „ ...	„	25 hours	7	8
25	887	200 „ ...	„	25	7	8
26	886	60 „ ...	„	49	6	8
27	877	100 „ ...	„	54	6	8
28	879	50—60 „ ...	„	84	2	5
29	859	200—300 „ ...	„	91	3	7
30	826	50 „ ...	„	102	3	7
31	854	Very few—0·1 c.c. ...	„	4½	5	9
32	854	Few „ ...	„	5½	4	9
33	842	A few „ ...	„	16	10	26
Group V. Sterile.						
34	885	Sterile—0·1 c.c. ...	„	2 hours	9 days	9 days
35	872	„ „ ...	„	4	5	5
36	788	„ „ ...	„	15	8	8
37	822	„ „ ...	„	79	6	9
38	822	„ „ ...	„	103	5	9
39	822	„ „ ...	„	127	4	9
40	819	„ „ ...	„	6 days	13	19
41	819	„ „ ...	„	7	12	19
42	796	„ „ ...	„	7½	5	12
43	819	„ „ ...	„	8	11	19
44	884	„ „ ...	„	8	3	11
45	797	„ „ ...	„	16	6	22

present in the blood 17 days before death and they continued to be present till at least 12 days before death occurred. This case also exemplifies the type of septicaemia referred to in the account of the preliminary series as "irregular" or "fluctuating" (compare case 18, Table II, vol. VI. p. 527 of previous paper).

Table II gives in a convenient form the results of a considerable number of examinations of blood arranged with reference to individual samples. The samples have been distributed into 5 groups, according to whether the colonies which developed from 0.1 c.c. of blood were "very numerous," "numerous," "fairly numerous," "few" or none. Further, the samples in each group are set forth in a sequence which has reference to the time, reckoned from the hour of death, when the specimen was taken.

No. 6 is noteworthy as illustrating the fact that a marked septicaemia may be present a considerable period before death,—in this case 72 hours. Nos. 21 and 22 deserve special notice since they are examples of septicæmic cases which ended in recovery. Bacilli were present in the blood of both (10 colonies per c.c. and 500—600 colonies per c.c.) 2 days after the reputed date of attack.

TABLE III. *Showing averages of "number of hours before death" and of "days of illness" for each Group in Table II.*

Group	Colonies of <i>B. pestis</i>	Hours before death	Days of illness
I	Very numerous	19.7 hours	6.4 days
II	Numerous	22.6	4.6
III	Fairly numerous	85.4	10.3
IV	Few	105.4	11.0
V	None	132.5	12.6

An average has been struck for each group of the number of hours before death when the specimens were taken, and similarly of the estimated number of days the illness lasted. The results are shown in Table III. From the figures presented therein two conclusions seem warranted, namely (1) that the degree of septicaemia bears a definite relation to the period before death at which it is determined, and (2) that the degree of septicaemia bears a definite relation to the acuteness of the illness.

Lastly, the tables clearly confirm the judgment we passed in the account of the earlier series on the value of the microscopical examination of the blood, to the effect that this method of examination is quite untrustworthy as an index of the severity of septicaemia which may be present.

PART II. ON THE INFECTIVITY OF THE URINE AND FAECES IN
HUMAN PLAGUE.

The following account summarises the work of previous observers on this subject.

Wilm (1897) stated that he found *B. pestis* in the *urine* 4 to 6 weeks after the cessation of febrile symptoms. The Austrian Plague Commission (1900) attempted the cultivation of *B. pestis* from the urine of 17 cases post mortem; they succeeded in 5 cases. They made fairly numerous attempts to cultivate the bacillus from the urine of patients before death but never succeeded. The German Plague Commission (1899) obtained a pure culture of *B. pestis* from the urine of only 2 patients. Most of their attempts at cultivation either from patients or at the post mortem yielded no results, the cultures remaining sterile or containing adventitious bacteria. The Indian Plague Commission (1901) examined 60 specimens of urine by cultivation methods, but were able to isolate *B. pestis* in only 3 cases. Tidswell (1900) examined the urine of 29 cases by cultural and inoculation tests but in every instance failed to prove that plague bacilli were present.

With regard to the examination of *faeces*, the Austrian Plague Commission investigated 8 cases in the post-mortem room in addition to fairly numerous cases before death. They never succeeded in demonstrating the presence of the plague bacillus by cultural methods. The plan of inoculating guinea-pigs cutaneously, which they brought into prominence after their return to Vienna, was not employed by them in Bombay. The German Plague Commission using cultural and animal tests were also unsuccessful. The faeces were examined by the Indian Plague Commission in 4 cases but without success. Tidswell (1900) by means of plates and by microscopical examination investigated the faeces of 20 cases with uniformly negative results.

This brief review of the literature of the subject makes it plain that unusual difficulties surround the examination for plague bacilli of the urine and faeces.

We decided to attack the problem on somewhat different lines from those pursued by our predecessors. Cultivation methods were entirely dispensed with, and animal tests were substituted for them. The guinea-pig, on account of its extreme susceptibility, was used as the experimental animal throughout.

Four series of experiments were carried out, namely:—

I. The cutaneous inoculation of graduated quantities of urine.

II. The cutaneous inoculation of faeces.

III. The subcutaneous inoculation of 1 c.c. of urine with, at the same time, the cutaneous inoculation of a control guinea-pig with 0.1 c.c. of the same sample of urine.

IV. Contact experiments in a flea-free godown with the soiled linen of fatal cases.

Our methods were as follows:—

Series I. After blood had been withdrawn from the patient in the manner already described, a Jacques' catheter, previously sterilised by boiling, was passed into the bladder and the urine allowed to flow directly into a sterile test-tube. The subsequent examination was carried out in a room adjoining the ward within a few minutes after the withdrawal of the urine. 1 c.c., 0.1 c.c. and 0.01 c.c. of each specimen of urine were measured off into three sterile watch-glasses by means of a graduated diluting pipette. After being made up to a convenient bulk with sterile broth each quantity was injected into the subcutaneous tissues of the thigh of each of three guinea-pigs. If microscopical examination showed that numerous bacilli were present in the blood higher dilutions were made, but this was rarely found necessary. The guinea-pigs after inoculation were returned to the laboratory, where they were kept under observation. In a few cases only the animals died as the result of the entrance into the tissues of organisms other than plague; on the whole the method proved quite satisfactory.

Series II. After the urine had been withdrawn from the patient, a sterilised glass tube (of about 6 mm. diam., except at one end where it narrowed in a slightly cone-shaped manner) was passed into the rectum as gently as possible so as to avoid accidental abrasions. As a matter of fact, probably from abrasions of the congested mucous membrane of the rectum caused by the insertion of the tube, the faeces were sometimes found to be blood-stained. We were generally able by this method to obtain a quantity of faeces sufficient for a test. Two guinea-pigs were used for every specimen of faeces examined, the faeces being well rubbed into a slightly scarified area of skin on the animal's abdomen.

Series III. 1 c.c. of the freshly drawn urine was injected subcutaneously into the thigh of a guinea-pig. At the same time 0.1 c.c. of the same sample was well rubbed into a shaved and scarified area about $\frac{1}{2}$ inch in diameter on the control guinea-pig's abdomen.

Series IV. Fifteen guinea-pigs were confined in a flea-proof godown in the laboratory compound. The bedding, recently soiled by the excreta of acute cases just before death, was added daily, each lot of bedding being kept in the godown for 48 hours.

Our observations and the results of the experiments in Series I and II have been tabulated in Table IV, in groups which make it easy to compare the four essential points: (a) infectivity¹ of urine, (b) infectivity of faeces, (c) quantitative estimation of the septicaemia and (d) microscopical examination of the blood.

It will be seen that the blood and urine of 27 patients were examined and that the urine of 8 was infective, *i.e.* 29·6%. On 7 occasions out of 22 on which a growth of *B. pestis* was obtained from the blood, the urine proved at the same time to be infective, *i.e.* the urine was infective in 31·8% of the septicaemic cases. In No. 8 no culture was obtained from the blood and yet 0·01 c.c. of the urine killed a guinea-pig. The explanation suggests itself that this was an instance of a diminishing septicaemia. In 15 instances in which a culture was obtained from the blood, the urine proved to be non-infective. The urine of No. 3 was highly infective,—0·0001 c.c. killing a guinea-pig of plague. This was associated with a marked septicaemia and the case is notable also as providing an example of a rare event in our experience—the presence of very numerous *B. pestis* on microscopical examination. The samples were taken 2 hours before death. It is evident from a study of the table that most of the cases whose urine proved to be infective had a severe septicaemia at the time of examination. This conclusion is warranted not only from the result of the cultural tests, but from the result of the microscopical examination, as may be seen from a comparison of Groups II and III in the Table. We may state, therefore, that when the urine is infective, the degree of infectivity stands in a direct relation to the degree of septicaemia. On the other hand, it would appear that at the moment of examination the blood may contain many bacilli and yet the urine may be non-infective. This receives illustration in Nos. 9, 10, 17, 18, 19, 22 and 23.

A comparison of Groups II and III from the point of view of the number of hours before death the samples were examined reveals a striking difference. This comparison shows that the most highly infective samples of urine were examined within 5 hours of death,—the interval before death being much longer in the case of the samples of Group III. The numbers in each group are admittedly small, but the

¹ For the sake of convenience we will employ the term “infective” as indicating that 1 c.c. or less of the urine killed a guinea-pig with plague, and the term “non-infective” to indicate that the guinea-pig was not affected by the inoculation of this amount. A similar meaning is attached to the terms in the case of the faeces. Again for the purpose of the present paper the term “septicaemia” may be allowed to mean that a growth occurred in 0·1 c.c. of blood.

TABLE IV. Giving details of the examination of samples of blood and excreta from cases of plague, which proved fatal.

		Series I and II.					Time reckoned from date of attack of illness
Serial Hospital no.	Quantitative estimation of septicaemia	Microscopical examination of blood	Smallest amount of urine infective	Infectivity of faeces	Time reckoned from hour of death		
Group I. (Urine, faeces, culture and microscopical examination of blood positive).							
1	771	A few colonies—0·1 c.c.	A few	1 c.c.	1 g.-pig died of plague faeces, blood-stained	14 hours	2 days
Group II. (Urine, culture and microscopical examination of blood positive).							
2	711	Uncountable colonies—0·1 c.c. ...	A few	0·1 c.c.	Negative	1½ hours	5 days
3	769	Thick layer—0·1 c.c. ...	Very numerous	<0·0001 c.c.	—	2	3½
4	775	“ “ “	A few	<0·01 c.c.	Negative	5	6
Group III. (Urine and culture positive—faeces negative).							
5	765	A few—0·1 c.c. ...	Negative	1 c.c.	Negative	25 hours	7 days
6	845	Uncountable isolated—0·1 c.c. ...	“	1 c.c.	“	19	6
7	826	A fine layer—0·1 c.c. ...	“	1 c.c.	“	54	7
Group IV. (Culture negative—urine positive).							
8	738	Sterile ...	Negative	<0·01 c.c.	Negative	21 hours	10 days
Group V. (Culture and microscopical examination positive—urine and faeces negative).							
9	743	Many isolated colonies—0·1 c.c. ...	A few	Negative	Negative	25 hours	5 days
10	784	Just isolated colonies—0·1 c.c. ...	A few	“	“	11	4

Group VI. (Culture positive—urine and faeces negative).

		Negative	Negative	47 hours	3 days	4 days
11	778	50 colonies per c.c. ...	Negative			
12	842	Very few colonies—0.1 c.c. ...	Negative			
13	708	A few colonies—0.1 c.c. ...	"	17 days	8	26
14	842	"	"	8 hours	7	7
15	843	"	"	12 days	13	26
16	778	About 500 per c.c. ...	"	9 hours	7	8
17	764	2000 per c.c. ...	"	23	4	4
18	851	Uncountable—0.1 c.c. ...	"	Just before death	6	6
19	817	Numerous	"	48 hours	9	11
		A fine layer	"	10	6	6

Group VII. (Culture positive—urine negative, faeces not tested).

		Negative	Negative	50 hours	6 days	8 days
20	887	20 colonies per c.c. ...	Negative		Unknown	Unknown
21	727	A few—0.1 c.c. ...	"	22		
22	786	Very many isolated colonies—0.1 c.c. ...	"			
23	783	"	"	12½	7 days	7 days
		"	"	9½	3	3

Group VIII. (Urine, faeces, culture, microscopic examination—all negative).

		Negative	Negative	1½ hours	6 days	7 days
24	765	Sterile—0.1 c.c. ...	Negative	14 days	11	26
25	842	"	"	96 hours	7	11
26	851	"	"	12	7	7
27	671	"	"	45	9	10
28	683	"	"	46	Unknown	Unknown
29	794	"	"	75	"	"
30	801	"	"	51	"	"
31	801	"	"	27	"	"
32	801	"	"		"	"

Group IX. (Urine, culture, microscopic examination negative (faeces not tested)).

		Negative	Negative	1½ hours	Unknown	Unknown
33	756	"	Negative	3½		
34	801	"	"	22	"	"
35	794	"	"	7 days	3 days	10 days
36	798	"	"			

The sign < indicates that a smaller amount than the amount stated was not inoculated so that the limit of infectivity may not have been reached. The sign — indicates that no test was made.

deduction, that the maximum infectivity of the urine occurs within a few hours of death, is probably not far wide of the mark, since this result doubtless depends on the fact that the septicaemia tends to be greatest at this time, and also on the fact that the tissue changes in the kidneys, as in other organs, progressively increase till death.

Proceeding now to the consideration of the infectivity of the faeces, it may be remarked that on one occasion only did a guinea-pig die as the result of inoculation. Even in this case we are not satisfied that the faeces were truly infective, because they had the appearance of being stained with blood from an accidental abrasion of the rectum. It may be added that the urine of this patient was not highly infective and that the septicaemia was not very severe. The blood and faeces of 20 patients were examined. We may say then that the faeces were not infective in at least 95 % of the patients examined. In 16 instances, in which the faeces were examined, the corresponding specimens of blood yielded a culture,—in other words, assuming that the faeces of No. 1 were really infective, the samples of faeces were infective in only 6 % of the septicaemic cases. We are justified then in concluding that the urine possesses greater infective properties than the faeces judging from the results obtained by the methods used in these experiments.

The urine of No. 3, Table IV, contained numerous plague bacilli and it is possible that in such a case the attendants might be in danger of contracting plague pneumonia from the spraying of plague bacilli in the air during the act of micturition.

It is necessary now to consider the bearing of these results related above on the question of the danger of infection from contact with excreta of plague cases under natural conditions. We do not think that any conclusions can be drawn from the experiments with urine in Series I as to the infectivity of the urine in relation to the spread of the epidemic, because the method of subcutaneous inoculation is a method which cannot occur in nature except from some untoward accident. The experiments are of interest, however, since they throw light upon the relation of the septicaemia to the bacilluria and since they show that in nearly 30 % of the cases examined the urine contained virulent *B. pestis*. It would appear from the experiments with faeces that the infectivity of the faeces is very slight and that, therefore, they cannot be regarded as a source of danger in the spread of plague. In order to imitate more closely a conceivable mode of infection by urine in nature, the experiments in Series III and in Series IV were carried out. In the former

TABLE V. Giving details of cases whose urine was proved to contain *B. pestis*.

Series III and IV.								
Hospital no.	Date sample was taken	Time before death sample was taken	Time after date of attack sample was taken	Duration of illness	Result of test (1 c.c. subcutaneously)	Soiled linen tested in godown	Remarks	
		2½ hours	5 days					
304	27. iii. 07	2½ hours	5 days	5 days	G.-pig died in 8 days	Yes	Blood taken on 25. iii. 07 and 26. iii. 07 gave a fine layer of just isolated colonies.	
306	27. iii. 07	2½	5	5	“ “ 8 “	“	Blood taken on 26. iii. 07 gave 2000—3000 colonies per c.c.	
332	29. iii. 07 30. iii. 07 1. iv. 07	5½	6	6	“ “ 6 “	“	Samples taken on 29. iii. and 30. iii. were without effect on g.-pigs.	
342	30. iii. 07 1. iv. 07	48	4 ?	6 ?	“ “ 9 “	“	Urine taken 1 hour p.m. without effect on g.-pig	
348	1. iv. 07	11	4	4	“ “ 10 “	“		
351	1. iv. 07	20	?	?	“ “ 12 “	“		
382	4. iv. 07	2	4	4	“ “ 7 “	“		
286	23. iii. 07	4	?	?	“ “ 9 “	No	Blood examination. 3 or 4 <i>B. pestis</i> in each field of microscope. Thick layer of growth in each culture of blood taken on 23. iii. 07.	
325	27. iii. 07	3	?	?	“ “ 6 “	“		
341	30. iii. 07	17½	4	5	“ “ 8 “	“		
365	4. iv. 07	29	6	7	“ “ 7 “	“		

TABLE VI. *Giving details of cases whose urine on testing was not proved to contain B. pestis.*

Series III and IV.					
Hospital no.	Date sample was taken	Time before death sample was taken	Time after date of attack sample was taken	Duration of illness	Remarks
284	28. iii. 07	21 hours	7 days	8 days	Soiled linen tested in godown. 40—50 colonies per c.c. in blood taken on 25. iii. 07.
289	27. iii. 07	9	8	17	Soiled linen tested in godown. No growth on 25. iii. 07 of blood. 50—60 colonies per c.c. on 26. iii. 07.
	29. iii. 07	7	10	17	
	30. iii. 07	6	11	17	
250	28. iii. 07	2 days	12	14	Soiled linen tested in godown.
301	27. iii. 07	5	5	10	Soiled linen tested in godown.
	28. iii. 07	4	6	10	
	29. iii. 07	3	7	10	
	30. iii. 07	2	8	10	
357	1. iv. 07	Just before death	2	2	Soiled linen tested in godown
358	1. iv. 07	4 days	2	5	Soiled linen tested in godown.
	2. iv. 07	3	3	5	
	3. iv. 07	2	4	5	
	4. iv. 07	19 hours	5	5	
274	23. iii. 07	?	?	?	400—500 colonies per c.c. in blood taken on 22. iii. 07. A fine layer of scattered colonies in blood taken on 23. iii. 07.
215	23. iii. 07	9 days	9 days after admission		No colonies in blood taken on 22. iii. and 23. iii.
292	27. iii. 07	7 hours	5 days	5 days	No colonies in blood taken on 26. iii.
309	27. iii. 07	4 days	5	9	
	28. iii. 07	3	6	9	
	30. iii. 07	1	8	9	
328	29. iii. 07	44 hours	5	7	
	30. iii. 07	20	6	7	
340	30. iii. 07	1 hour	?	?	
349	1. iv. 07	2 hours	6 days	6 days	
371	4. iv. 07	2 days	5	7	
372	4. iv. 07	2½ hours	?	?	
378	4. iv. 07	24	4 days	5 days	
283	23. iii. 07	—	4	—	Recovered.
314	28. iii. 07	—	5	—	Recovered. 20—30 colonies per c.c. in blood taken on 26. iii. 07.
	29. iii. 07	—	6	—	
362	2. iv. 07	—	5	—	Recovered.
239	27. iii. 07	—	—	—	Under treatment, 24. iv. 07.
313	27. iii. 07	—	—	—	" " "
	28. iii. 07	—	—	—	
344	1. iv. 07	—	—	—	" " "
	3. iv. 07	—	—	—	
352	1. iv. 07	—	—	—	" " "
356	3. iv. 07	—	—	—	
327	28. iii. 07	—	—	—	" " "
	29. iii. 07	—	—	—	
	30. iii. 07	—	—	—	
	2. iv. 07	—	—	—	
350	1. iv. 07	—	—	—	" " "

series, with a few exceptions, the examination of the blood was omitted; the samples were, however, invariably taken from patients who were acutely ill of the disease. The results are tabulated in Tables V, VI and VII. The experiments in these series were purposely carried out in order to compare the infectivity of the urine by cutaneous inoculation—a method comparable to a conceivable mode of infection in nature—with the results obtained by subcutaneous inoculation,—a method which, as we have said, can hardly occur in nature.

We may note that 57 samples of urine, derived from 37 cases, were examined. Eleven samples (19·3%) killed the guinea-pig when inoculated subcutaneously, but in no single instance did a control animal inoculated cutaneously contract the disease. This result is very important, because it shows that a sample of urine, although it contains virulent *B. pestis*, may fail to give plague to a susceptible animal when rubbed into an abraded area of skin. It is interesting to note also that the guinea-pigs inoculated subcutaneously took an unusually long time to die, the average duration of life of the test animals working out at 8·2 days. Whether the result was due to a diminished virulence of the bacilli or to small numbers of bacilli being present in the urine, we are unable to say.

TABLE VII. *Additional cases in which the soiled linen was tested in the godown; urine not tested separately.*

Series IV.

Hospital no.	Duration of illness	Remarks
260	6 days	Thick layer of growth in blood taken; just before death <i>B. pestis</i> fairly numerous microscopically.
285	7	A few colonies in blood taken 4 days and 2 days before death and about 500 colonies per c.c. of blood taken 24 hours before death.
369	4	
392	3	
409	5	

The details of the cases which furnished soiled linen for the experiments in Series IV will be found in Tables V, VI and VII. As already stated, each lot of linen was kept in the godown for 48 hours. The first lot of bedding was put in on 23rd March 1907 and the last on 9th April 1907, so that the 15 animals were exposed continuously to contact with the excreta for a period of 18 days. In all 18 lots of bedding were put into the godown. In 7 cases the bedding was from patients whose urine contained virulent *B. pestis*, as proved by the death of a guinea-pig after subcutaneous inoculation of the urine.

In 4 instances in addition to these, the bedding was taken from septicæmic cases. Nearly all the cases were acute and all were fatal; the bedding soiled by the patient just before death was in every instance selected for the experiment. In spite of the intimate contact with this material in a confined space none of the guinea-pigs contracted the disease.

Reference may be made here to certain experiments already described (vol. VII. p. 380), in which many of the samples of urine examined during the course of the present experiments were used to contaminate the food of Bombay rats. Even when the urine proved infective, in not a single instance did the rat suffer any harm. We may therefore conclude that little danger exists to rats of a nasal or mouth infection from contact with excreta of plague patients.

We have finally to record our thanks to Dr Khan Bahadur N. H. Choksy, M.D., Medical Officer of the Maratha Hospital, for his courtesy and kindness in placing the clinical material at our disposal and for giving us accommodation and facilities for carrying out the work at this hospital.

CONCLUSIONS.

1. A severe septicaemia may be present at a comparatively early stage of the disease and for a considerable number of hours before death.
2. The degree of septicaemia as a rule stands in an inverse relation to the interval before death the observation is made, *i.e.* the shorter the interval before death, the greater is the septicaemia.
3. The degree of the septicaemia stands in a direct relation to the acuteness of the illness.
4. The septicaemia is usually of a progressive type, but is occasionally of a "diminishing," "irregular" or "fluctuating" type.
5. A patient with a septicaemia may recover.
6. Microscopical examination of the blood cannot be regarded as a trustworthy index of the degree of septicaemia.
7. The urine of nearly 30% of the cases in Series I and in 19·3% of Series III contained virulent *B. pestis*, which killed test animals when inoculated subcutaneously.
8. When the urine proves to be infective by the subcutaneous method of inoculation, its degree of infectivity is directly related to the degree of septicaemia.

9. The maximum infectivity of the urine, as tested by the subcutaneous inoculation with guinea-pigs, appears to occur within a few hours of death.

10. At a particular stage of the disease an absence of infectivity of the urine may coexist with a severe septicaemia.

11. The urine may be infective although at the time of testing a septicaemia is not present.

12. Experiments devised with the object of testing the infectivity of the excreta from the point of view of the spread of the human epidemic support the conclusion that the excreta of plague patients are ineffective in this regard. These experiments show

(a) that the faeces are rarely infective even when a septicaemia is present:

(b) that the urine—in some cases containing virulent plague bacilli—from patients acutely ill of the disease failed to infect guinea-pigs when rubbed into a scarified area of skin:

(c) that guinea-pigs exposed to intimate and prolonged contact with linen soiled with the excreta of moribund patients remained free from infection.

REFERENCES.

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XXIX. OBSERVATIONS ON THE BIONOMICS OF FLEAS
WITH SPECIAL REFERENCE TO *P. CHEOPIS*.

- I. Development, length of life and breeding.
- II. Relation to hosts.
- III. Dispersal.
- IV. Collection and examination.

FLEAS (*Aphaniptera*, *Siphonaptera*) are wingless insects which run or hop about. They are for the most part parasitic in their habits, being found only on the homoiothermic vertebrates, especially on those which build nests or have more or less fixed haunts or homes. Like the majority of other parasites they have the body flattened, but differ from most parasitic insects in that this flattening is in a lateral direction, so that the transverse diameter of the body is small and the vertical diameter great. Attention has already been drawn to certain other morphological peculiarities of these insects (vol. VII. p. 446).

I. DEVELOPMENT, LENGTH OF LIFE AND BREEDING.

Fleas undergo a complete *metamorphosis* in their development. The imago, perfect or adult female flea lays eggs. These find a resting place either on the ground or on some other suitable object, but are not attached to the fur of the host upon which the flea feeds.

The *eggs*. The eggs are said to be laid at all seasons, an observation which has been confirmed by us as regards *P. cheopis* in Bombay and in the Punjab. They are about as large as a small pin's head, ovoid or round in shape and of a waxy white or pearly colour. From one to five eggs, one after the other, are laid at a time. They generally hatch in a few days, in Bombay in about two days, after they have been deposited.

The *larva*. From the egg a worm-like larva emerges. It is furnished with chewing or biting mouth parts and lives upon almost any kind of

refuse, animal or vegetable. The larvae are most easily detected among sand or bran in which the eggs have been laid by shaking these materials and watching for movements among the particles. They will be noted as minute, white, caterpillar-like creatures, endeavouring to conceal themselves from the light. They are liable to be confused with, and must be carefully distinguished from, the somewhat similar larvae of the Mycetophilidae, a family of minute flies, and also from the larvae of certain grain-eating weevils.

The larva is made up of a series of well-defined segments, fourteen in number including the head. Each segment is furnished with a number of hairs or bristles which are found on the ventral, dorsal and lateral surfaces of each segment except the first and last. The hairs on the dorsal surface of the penultimate segment are especially noteworthy, inasmuch as their length varies in the different species. They are, for example, remarkably long in the case of the larvae of *P. cheopis*, while they are comparatively short in the case of the larval human flea. On the ventral surface of the last segment two minute finger-like processes will be found: these are apparently used in progression.

The *pupa*. After a varying period, not less than a week, the larva, when full grown, becomes sluggish, ceases to eat and makes a cocoon. The cocoon is spun with fine, white, silk-like fibres and its surface is frequently covered over with dust, bran or other small particles of rubbish which adhere to the fibres.

The *imago*. The perfect flea escapes from the cocoon after a period of from seven to fourteen days. The young flea has now to seek for a host, for it is capable of living only on blood; its mouth parts, as we have already seen (vol. VI. p. 486), are so constructed that it can only feed on liquid food. Young fleas, that is to say those just escaped from the cocoon, are, however, endowed with a considerable amount of vitality, for they can live without food for from seven to fourteen days. Older fleas, which have sucked the blood of a host, die within a week if deprived of food.

The length of life of fleas.

With a view to finding how long *P. cheopis* could survive without food in different circumstances, we devised a number of experiments, in which these fleas were placed in various materials and we noted how long they were able to live in these surroundings. In one series of experiments (Table I) 150 fleas were added to each of the following

materials and the time when all, or nearly all, the fleas were dead was noted. They were kept without a host.

TABLE I.

Serial number of the experiment	Material in which the fleas were placed	Number of days the fleas survived
1	Bran	All dead in 6 days.
2	Bran	Ditto.
3	Bran with moisture	All dead in 7 days.
4	Cotton rags	All dead in 6 days.
5	Gunny bags or sacking	Ditto.
6	Rice and pulse	Ditto.
7	Sand, with moist cowdung in one portion of the box	15 alive on 6th day.
8	Ditto	3 alive on 8th day.
9	Ditto	All dead on 11th day.
10	Ditto	All dead on 14th day.
11	Ditto	4 alive on 11th day.
12	Ditto	1 alive on 13th day.

From these experiments it is obvious that *P. cheopis* is unable to live for many days in the absence of a liquid food supply. Some of the experiments, especially 11 and 12, show that in a box containing sand, but with a pat of moist cowdung in one portion, fleas could survive for a longer period than in circumstances in which they had not access to any moisture. In any case in the absence of their natural food they were unable to live for a fortnight.

We next endeavoured to find how long rat fleas could live when supplied with their natural food, the blood of a rat. In this experiment 36 fleas were placed in the usual glass box along with a rat on which they could feed. The number of fleas surviving on this rat and in the sand contained in the box was noted from time to time. Care was taken to change the glass box and the sand it contained approximately every tenth day, so that the count would not be vitiated by the development of new insects from eggs laid by the original fleas placed in the box. The details of the experiment are given in Table II.

TABLE II.

Date	Day of experiment	Number of live fleas found	Percentage alive
20. v. 06	1st day	36	100
30. v. 06	10th „	15	42
9. vi. 06	20th „	8	22
18. vi. 06	29th „	2	6
30. vi. 06	41st „	2	6
10. vii. 06	51st „	0	All dead.

From this experiment it will be seen that *P. cheopis* can live for at least 41 days when its food supply is derived from a rat. A similar series of six experiments (Table III) was carried out, in which a guinea-pig was used as the host for the rat fleas in place of a rat, with the following result:—

TABLE III.

Day of experiment	Number of fleas found	Percentage alive
1st day	240	100
5th ,,	114	48
10th ,,	41	17
20th ,,	3	1

Again, another series of experiments was made to ascertain how long rat fleas could live on man as a host. This series was carried out in the following way. Forty fleas were put into a long, wide-mouthed, glass bottle which contained some sterile sand in the bottom. Twice daily, for 15 minutes each time, a man's hand and arm were introduced into the bottle. The fleas hopped on to the hand and sucked the man's blood. Those which crawled up the arm were gently returned to the bottle. The fleas were caught and removed to a fresh bottle with fresh sand approximately every tenth day to avoid multiplication by breeding. From the subjoined details (Table IV) of the experiment it will be seen that *P. cheopis* can live for 27 days when fed on human blood alone.

TABLE IV.

Day of experiment	Number of fleas alive	Percentage alive
1st day	160	100
22nd ,,	9	6
25th ,,	5	3
27th ,,	1	1
28th ,,	0	All dead.

If we compare the figures in Table II with those in Table IV we note that while 6 per cent. of the fleas were alive on the 41st day when they were fed on a rat, the same percentage, namely 6 per cent., were alive only up to the 22nd day when they were fed on man. Again, all the fleas were dead on the 28th day when fed on man, but two out of 36 fleas were still alive on the 41st day when they were fed on a rat. It is evident, then, that rat's blood is a more suitable food than human blood for rat fleas.

Certain points of difference require to be noted in the behaviour of rat fleas when fed on the rat and when fed on man :—

(1) It was noted that the fleas were much more readily attracted by the rat than by man.

(2) Although the fleas jumped on the man's hand they took some time to begin to feed. They crawled about and seemed to have some difficulty in selecting a suitable spot on which to begin their sucking operations.

(3) The fleas much more readily fell off the man's arm, when he moved it, than they did when the rat moved; they were able to get a firmer hold on the rat than on man. It is interesting to note in this connection the larger claws of the human flea.

The time necessary for the completion of the cycle of development.

We have attempted to determine experimentally the time required for the completion of the cycle of development from the egg to the imago. We found that this time varied, one of the chief conditions which caused the variation being the nature of the food supply of the larva. Under the most favourable conditions from 21 to 22 days were necessary for the completion of the cycle, as the following experiments show.

Some sand mixed with guinea-pig droppings was placed in a glass box. Care in the first instance was taken to ensure that no fleas had had access previously either to the sand or to the droppings, so that these substances contained no flea's eggs. Into boxes of this description a number of pregnant female fleas, together with some males, were placed. The fleas were not supplied with a host so that within a week they were all dead. The cages were now carefully observed for the development of adult fleas, with the result that in three boxes these appeared in 21 days and in two cages in 22 days. In another series of experiments a single female was placed in a small entomological box containing a similar mixture of sand and rubbish as was used in the previous experiment. In two of these boxes adult insects were found on the 21st day. In other experiments, where larvae were fed on bran alone, adult fleas developed at intervals varying from four to six weeks.

Climatic conditions, also, appear to affect the development of some species of fleas. Thus, we found in the Punjab that, while *Ceratophyllus fasciatus* could be captured on rats from the beginning of November throughout the winter months, they began to disappear in

the beginning of April, and only one isolated flea of this species, the last of the season, was taken on a rat on the 15th of May. From this date to the beginning of November these fleas had entirely disappeared. In what form, egg, larva, pupa or perfect flea, this species tided over the hot and rainy seasons we were unable to ascertain.

Breeding places of fleas.

Fleas are most abundantly found in the haunts of their hosts, owing to the fact that the houses or nests of the hosts are par excellence the breeding places of these insects. Here eggs, larvae, pupae and adult fleas are unusually numerous. The breeding places of the different species of fleas, of course, vary with the habits of their host. Thus, birds' nests are the breeding places of fleas found on birds, human habitations the breeding places of *P. irritans*.

In the case of rats the breeding places of their fleas vary with the habits of the species of rat; thus, those found on *M. decumanus* breed in the burrows made by this rat, those on *M. rattus* in all sorts of situations which afford a shelter to these animals. We have on several occasions examined the earth and rubbish collected by *M. decumanus* in their burrows, where their young ones are found, and have noted the presence of a large number of adult and larval fleas. A nest made by *M. rattus* on a grain bag was also examined and a number of larvae of *P. cheopis* was found in the sacking in its neighbourhood. Where a trade is carried on in gunny bags this means of distributing *P. cheopis* should be kept in mind.

Dampness in the surroundings of a breeding place hinders the development of the insects. Adult fleas, also, appear to dislike wetness. A number of our failures in our early experiments to transmit plague from animal to animal by means of fleas, and possibly in the experiments of others, can be attributed to this cause, viz., the death of the fleas because of the wet surroundings. This difficulty was overcome by placing a layer of sand in the cages containing the experimental animals. The sand served to keep the cages dry and allowed the fleas to live and indeed to multiply. Long glass boxes with a layer of sand in the bottom in which a white rat or a guinea-pig was confined served as excellent breeding places for fleas.

Breeding season of fleas.

The question of the seasonal prevalence of fleas is discussed in full below. We propose now, however, briefly to record some observations which were made in the laboratory on the breeding season of fleas and on the effect of a high mean temperature on breeding.

(a) *Observations on the breeding season of P. cheopis in Bombay.* These experiments were carried out with the view of ascertaining if there was a definite season of the year at which this species of flea breeds, and if there was a season at which breeding does not take place. The method employed was as follows. In a long flea-proof cage of the same pattern, but much larger, as that figured in the first report (vol. VI. p. 435 and Plate IV), a wild rat was either allowed to run free or placed in a wire cage inside the glass cage. Forty rat fleas, taken from rats caught in Bombay, were placed in the cage along with the rat. A cage of this description was prepared every month from February 1906 to February 1907. The cages were all kept at laboratory temperature. Each week a census of the fleas in each cage was made in the following manner. The rat was removed from the cage and the fleas taken on it were enumerated, the latter being kept in a test tube. The rat was put back into the cage and left there for half an hour. It was then removed and the fleas on it again counted. This process was repeated a third time. The total number of fleas got on the three counts was taken as the census number; at the end of the operations the rat and the fleas were returned to the cage.

An analysis of the results, which it is unnecessary to reproduce, showed that breeding took place in one or more of the cages at all seasons of the year. While this was so, it would appear that in the month of June breeding was not so vigorous as during the rest of the year. From these observations, therefore, we can conclude that under the conditions of the experiments, which, it is to be remembered, were quite artificial, *P. cheopis* can breed at any season of the year in Bombay. It would be fallacious, however, to draw from these results any conclusion as to what occurs in nature.

(b) *Observations on the effect of temperature on the breeding of P. cheopis.* Several series of experiments with this object in view were carried out simultaneously (1) at room temperature, namely 75° to 80° F.; (2) in a specially constructed hot room, the temperature of which was kept at from 88°—90° F.; and (3) in a specially constructed

cool room, the temperature of which was maintained at about 72° F. These observations may be detailed as follows:—

Series 1. This series was designed to ascertain if a high mean temperature influenced the development of the flea from the egg to the perfect insect. The technique was as follows: Into each of eight flea-proof cages with sand at the bottom there were placed a rat and 40 fleas. At the end of 24 hours the rat was removed and the sand with those fleas not on the rat tipped out on to a tray, such as is described below (p. 256). The fleas were carefully removed and the sand replaced in the cage. There were, therefore, only eggs left in the sand. The cages were now divided into two lots, four cages kept at room temperature (75°—80° F.) and the other four in the hot room (88°—90° F.). At the end of three weeks the cages were opened and the fleas enumerated by means of the tray in the manner to be described. In the cages kept at room temperature, 21 adult fleas were found; in those kept in the hot room, none.

Series 2. This series of observations was made with the same object in view as that just described, but the technique was somewhat different. Into a long flea-proof cage, supplied as usual with sand in the bottom, a wild rat and forty fleas were placed. The rat and fleas were left in the cage. Some of the cages were kept at room temperature and an equal number in the hot room at 88°—90° F. At the end of three weeks, four weeks, five weeks and six weeks, the fleas were enumerated in the usual manner. In all, five cages at each temperature were treated in this way. The combined results of the flea census for each week is given in Table V.

TABLE V.

No. of fleas taken after	Room temperature (75—80° F.)	Hot room (88—90° F.)
3 weeks	86	23
4 „	329	57
5 „	527	72
6 „	456	68

It would appear then from this table that breeding went on at both temperatures, but much more vigorously at the lower temperature than at the higher.

Series 3. This series of experiments was made with the object of ascertaining if a high mean temperature had any effect on the imago as regards the laying of eggs. The following was the technique:—Twenty young fleas, which had been bred in the laboratory, were placed along with a guinea-pig in a flea-proof cage. One such cage

was kept at room temperature and another, prepared in exactly the same manner, in the hot room. At stated intervals between the third and eleventh day the guinea-pig was removed from the cage and the fleas found on it placed in a test tube, which was now kept for two hours at the same temperature at which the cage had been kept. The fleas were then put back into the cage and the number of eggs deposited in the test tube were enumerated. The guinea-pig was also returned to the cage.

In all three pairs of experiments were made in this way. The following Table summarises the results:—

TABLE VI.

No. of days from beginning of experiment on which eggs were counted	Room temperature (75—80° F.)		Hot room (88—90° F.)	
	No. of fleas put into test-tube	No. of eggs deposited	No. of fleas put into test-tube	No. of eggs deposited
3	37	16	24	0
4	18	9	5	0
6	15	5	5	0
9	28	22	15	0
11	10	19	10	0

Series 4. The last series of experiments was made with the object of ascertaining if temperature had any effect on the development of the egg into the larva. The following was the method of experimentation:—Fleas recently taken from their host were allowed to remain in a test tube for from two to three hours. They were then removed, and the eggs which they had deposited were collected. The eggs were then divided into three batches, one of which was kept at room temperature, another in the hot room, and the third in the cold room. At the end of seven days the larvae which had developed out from the eggs were counted. Table VII contains the results of this series of experiments.

TABLE VII.

	Room temperature (75—80° F.)	Hot room (88—90° F.)	Cool room (72° F.)
No. of eggs put in	461	420	128
No. of larvae which hatched out	33	2	43
Percentage of larvae on eggs	7.2	0.5	33.6

From a study of all these series of experiments we must conclude that a high mean temperature affects the breeding of fleas to a considerable extent, that it appears not only to restrain the imago from depositing eggs but also to be deleterious to the development of the eggs into larvae. There would seem also to be an optimum temperature at which breeding takes place more vigorously than at other temperatures.

II. THE RELATION OF FLEAS TO THEIR HOSTS.

Distribution of fleas, geographically and on animals.

Fleas are found in all climates from the arctic regions to the tropical zones. With regard to the geographical distribution of particular species it appears that, while some species have a more or less universal geographical distribution, such as *P. irritans*, others are found in more or less limited areas, such as *Sarcopsylla penetrans*, the chigger or sand flea. Attention has already been drawn to the geographical distribution of rat fleas in a paper by the Hon. N. C. Rothschild in these reports (vol. VI. p. 483). He concludes his paper by stating that "his opinion with regard to the different species of fleas that are found on house and port rats all over the world is that, except in Northern and Central Europe, *P. cheopis* is the commonest rat flea and in some localities is almost the only flea found upon rats." Fleas from rats in Northern and Central America had, however, not been examined. It is sufficiently important to point out here that the same host may have one species of flea commonly living on it in one part of the world and a different species in another part. For example, *Ceratophyllus fasciatus* is the common flea found on *Mus decumanus* in Western Europe, where *M. decumanus* is the common rat, while in India on this rat *P. cheopis* is found. Again, while *P. cheopis* is practically the only flea found on *M. rattus* in Bombay, in the Punjab during the cold weather *Ceratophyllus fasciatus* is present to the extent of about 2% of the fleas found on this rat (see these Reports vol. VII. p. 916). It would appear, therefore, that *M. decumanus* is probably the true host of *Ceratophyllus fasciatus*, and *M. rattus* the true host of *P. cheopis*, but that climatic conditions (geographical position) may to some extent favour the presence of a particular species of flea.

How long fleas remain on their host. Fleas, unlike many parasitic insects, do not remain constantly upon their host. A considerable part of their existence is passed on the ground, generally in what may be termed the home or nest of the host. In this respect, however, considerable differences exist among different species of fleas. We need only remark that such a species as, for example, *Sarcopsylla penetrans*, inasmuch as the female burrows into the skin of its host, remains of necessity upon its host for the largest part of its life. On the other hand, the human flea, *Pulex irritans*, apparently visits its host only for the purpose of obtaining food, and after a

satisfactory meal leaves its host, probably to lay its eggs in a suitable environment for the development of the larvae. In this case a considerable part of the life of the flea is spent apart from its host.

Selection of particular hosts by fleas.

It will have been observed in the remarks made above that certain species of flea have been associated with certain species of host. So much is this the case, that in common parlance the host has occasionally given the name to the flea. Thus, we have spoken of the human flea when we refer to *Pulex irritans* and of the rat flea when we mean *Pulex cheopis*. In theory each species of flea has its own particular host, generally known as its true host. In practice, however, it is often difficult to define this host, especially for certain species of fleas, owing to the fact that the flea commonly found on one host is often found on another host of a different species.

Thus for example, *Pulex felis* has been found by us on the dog, the cat, the tiger, the panther, the goat, the horse, the rat, the hedgehog, the kangaroo, the deer, the guinea-pig, the rabbit, the monkey, and on man and some other animals. While this species of flea is found on such a wide range of animals, yet it has a distinct preference for particular hosts. If a number of these fleas were given the choice of a meal on, say, the blood of a dog, a man, or a guinea-pig, they would select these animals in the above order. There is, however, very little difficulty in defining the true host of some other species of fleas; the human flea, *Pulex irritans*, for example, is seldom found on any other host than man. We have rarely noted it on rats and guinea-pigs.

The selection exercised by a particular species of flea for a particular host has obviously an important bearing on the spread of plague among men if this is effected by means of the rat flea *P. cheopis*. We propose therefore to enlarge on this selective habit of fleas and we shall confine our attention to the selection exercised by three species of fleas, viz. *P. irritans*, *P. felis*, and *P. cheopis* for three particular hosts, viz. man, rat and guinea-pig. We shall record our observations on each species of flea separately under the following heads:

- (1) The animals on which the flea has been found by us:
- (2) Experiments with fleas confined in test tubes:
- (3) Experiments with fleas kept in large glass boxes:
- (4) Observations in houses.

A. *Pulex irritans*, the human flea.

1. *The animals on which P. irritans has been found by us.* This flea is very select in the choice of its host. We have found it almost exclusively on man. On one or two occasions only has a human flea been noted on a rat or on a guinea-pig.

2. *Experiments with P. irritans confined in test tubes.* When this flea is confined in a test tube and allowed to feed upon the body of a man, rat or guinea-pig through a piece of muslin placed over the mouth of the tube it readily feeds on any of these animals.

3. *Experiments with P. irritans kept in glass boxes.* We have already (vol. VII. p. 413) drawn attention to the fact that human fleas kept in boxes with rats or guinea-pigs as their food supply rapidly die out. We have seen that after 24 hours only 1·2 % of the fleas originally put in could be recovered and that after 72 hours this number was further reduced to 1 %.

4. *Observations in houses on P. irritans.* We had frequent opportunities for observing this species of flea in the houses we visited in Bombay, in some of which it was present in such large numbers that we had only to enter the houses for a few seconds to find our legs covered with them. On one occasion two guinea-pigs, placed in a house which had been vacant for some days and in which the fleas must have been short of food, failed to attract any of this species, while a man who entered the house shortly afterwards acted as an admirable trap. Using man as a trap for this flea we have been able to capture in vacated houses large numbers for experimental purposes, as the following examples show :—

In one house on April 19th the following fleas were caught on a man's legs, namely, 84 *P. irritans*, 8 *P. felis*, and 1 *Sarcopsylla gallinacea*. In another house on 20th April, 31 *P. irritans* were taken on a man's legs in a few minutes. Again, in a third house 150 *P. irritans* and four *P. felis* were captured in a short time. From the tanglefoot experiments already recorded (vol. VI. p. 479) it will be seen that, judged from the number of human fleas found on the tanglefoot, white rats attract *P. irritans*, but it is noteworthy that very few of this species were caught on the animals which were not protected with tanglefoot. It is possible that the rat attracted the fleas, but that after feeding upon its blood they left it to return to the room, where perchance they might find a more suitable host.

B. *Pulex felis*, the dog or cat flea.

1. *The animals on which P. felis has been found by us.* The long list of animals on which this flea has been found by us has already been given (p. 246).

2. *Experiments with P. felis confined in test tubes.* Cat fleas confined in test tubes and allowed to feed on the body of a rat, guinea-pig or man, through a layer of muslin placed over the mouth of the tube, readily sucked the blood of all three animals.

3. *Experiments with P. felis in glass boxes.* Cat fleas kept in a glass box, where they have access to a guinea-pig for a host, rapidly die out as the following table shows:—

TABLE VIII.

Number of fleas present on							
1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day
233	64	9	3	2	2	1	0

4. *Observations in houses on P. felis.* During our visits to plague infected houses we frequently came across rooms in which cat fleas were abundantly present. In some of the houses in Sion village we had to leave a room because of the large number of these fleas which attacked us. On one occasion in house I. 5. 12 in Sion Koliwada we found twelve cat and ten rat fleas on a dead plague infected rat. This, however, was an altogether unusual phenomenon, as cat fleas were rarely found on rats.

Only twelve cat fleas were captured on guinea-pigs placed in the houses of Sion village, although some of the houses were swarming with these fleas (vol. VII. p. 826). In a goat stand, where cat fleas were numerous, a man and a guinea-pig were simultaneously used to trap the fleas. A large number was captured on the man and only a few on the guinea-pig. It would appear then that man is selected in preference to the guinea-pig by the cat flea. Moreover, it is a common observation that a cat or a dog may be badly infested with fleas while man, living with these animals, does not suffer from the bites of the insects unless they are very numerous. The cat flea, therefore, prefers to feed on a dog or a cat rather than on man.

C. *The Indian rat flea (Pulex cheopis).*

1. *The animals on which P. cheopis has been found by us.* We have found *P. cheopis* on rats (*Mus rattus*, *M. decumanus*, *Nesokia bengalensis*), musk rats, guinea-pigs, cats, rabbits, an Indian antelope, a kangaroo, and on man.

2. *Experiments with P. cheopis confined in test tubes.* This flea readily sucks the blood of man, guinea-pig or rat when confined in test tubes and given the opportunity to feed on these animals.

3. *Experiments with P. cheopis kept in glass boxes.* We have already detailed experiments in which rat fleas were shown to have lived on man for 27 days, on a guinea-pig for 20 days, and on a rat for at least 41 days. We shall now record some experiments which illustrate the selection of certain hosts by the rat flea. For the purpose of these experiments in some cases we used long glass boxes in which rat fleas were breeding freely and in which the number of fleas varied considerably:—

(a) In a flea breeding cage, in which the fleas were numerous, we placed for an equal time a rat and a guinea-pig. The fleas found on the animals were counted. The first count gave 65 fleas on the rat, and 106 on the guinea-pig. The relative positions which the rat and the guinea-pig had occupied in the box were now reversed and the animals were again put into the box for a few minutes; they were taken out together and the fleas were counted on each animal. This second count gave 50 fleas on the rat and 38 on the guinea-pig. There appeared, therefore, to be little preference in either direction.

(b) In another flea breeding box, in which the number of fleas was great, the following experiment was carried out.

A *M. rattus*, which had been confined in the box for some days to supply food for the fleas, was kept in, while other animals were put into other portions of the cage. In the first place a man's hand was introduced for five minutes. In that time eight fleas had jumped on to it and five of them had bitten him, leaving marks where they had fed. A guinea-pig was next placed in the box for five minutes. It was then taken out and examined for fleas, 41 being captured. Thirdly a white rat was placed in the box for the same period; it was then examined for fleas, 24 being captured on it. Finally, the *M. rattus*, which had been present in the box during the whole experiment, was removed and on it 133 fleas were found.

(c) In another flea breeding box, in which the number of fleas was comparatively small, and in which as in the previous experiment a *M. rattus* had been present for some days to supply food for the fleas, the following experiments were carried out.

Experiment I. The *M. rattus* being present in the box, other animals were placed in it. First, a white rat was put in for five minutes. It was then taken out and 13 fleas were found on it. A guinea-pig was then placed in the box for five minutes and on it 18 fleas were caught. Lastly, a man's hand was introduced into the cage for five minutes, but no fleas came on it in this time. The *M. rattus* which had been in the cage throughout the experiment was then removed and examined for fleas, 69 being taken on it.

For the purposes of the following experiments the fleas, but not the rat, were returned to the box.

Experiment II. The fleas having been starved for 24 hours, a man's hand was introduced into the box for five minutes. One flea came on to it and bit him. Then a white rat was placed in the cage for five minutes; 14 fleas were caught on it at the end of that period. Lastly, a guinea-pig was put into the cage for five minutes and nine fleas were captured on it. All the fleas were returned to the box.

Experiment III. The fleas having now starved for 48 hours the same procedure was adopted as in the previous experiment. On this occasion one flea came on to the man's hand and filled itself with blood. On the rat 28 fleas were captured and on the guinea-pig 16 fleas.

In the above experiments we had no definite knowledge of the number of fleas present in each box as they were being used as flea hatchingeries. The number of fleas, therefore, varied to some extent from day to day, as new fleas were hatched out and old ones died off. In another series of experiments, which was carried out as follows, we used a fixed number of fleas.

(d) A large number of fleas which had just been taken off wild rats were placed in some sand. They were not supplied with any host to feed on. From this stock of fleas we were able to collect definite numbers, which had been starved for 24 or 48 hours, as they were required.

Experiment I, with fleas starved for 24 hours. Twenty fleas from the stock were placed in each of three boxes. A man's hand was introduced into one box, a guinea-pig into another and a rat into the third. The animals were left with the fleas for five minutes. On the man's

hand 10 fleas were captured, the other 10 fleas which had not fed being found in the sand in the bottom of the box. On the guinea-pig 16 fleas were caught, and on the rat 17 fleas.

Experiment II, with fleas starved for 48 hours. On this occasion 12 fleas were taken on the man's hand, 15 on the guinea-pig and 18 on the rat. It is noteworthy that in the case of the guinea-pig three fleas which had filled themselves with blood were found in the sand at the bottom of the cage. These fleas had apparently satisfied themselves and left their host within five minutes.

(e) In another experiment an attempt was made more nearly to simulate the conditions which occur in nature when a rat dies from plague and the fleas have left the corpse. In this case we placed 200 fleas, which had just been removed from live wild rats, in a glass box containing sand and introduced a man's hand into the box after certain intervals for the purpose of ascertaining how many fleas would attack him after they had been starved for different periods. The following observations were made:—(1) After the fleas had been starved for 24 hours, three fleas came upon the man's hand in five minutes; they all bit him. A large number of fleas were seen alive in the box but they did not go on to the man. (2) After the fleas had been starved for 72 hours a man's hand was introduced for five minutes. On this occasion a large number of fleas immediately jumped on to the hand and some of them crawled up the arm. These latter were gently brushed back into the box. At the end of the five minutes from 25 to 30 fleas adhered to the hand; most of these were feeding or had fed, but it was a matter of considerable difficulty to know exactly how many had fed. Many fleas were still alive in the sand. (3) After the fleas had been starved for 96 hours the same experiment was repeated. Again a large number of fleas at once jumped on to the hand and climbed up the arm. After five minutes 20 fleas, most of which had bitten the man, were found clinging to his hand. A fair number was still alive in the box. (4) After they had starved for 120 hours not many fleas were seen to be alive, but in five minutes six were found feeding on the man's hand, and soon their stomachs were seen to be full of freshly drawn blood.

4. *Observations in houses on P. cheopis.* We have already (vol. VII. p. 475) described some observations in which rat fleas were taken in large numbers on the legs of men who entered plague infected houses. We shall, therefore, confine ourselves now to certain experiments carried out in the godowns which have frequently been referred to (see vol. VI.

p. 450). Observations made in these buildings possess certain advantages over observations made in houses inasmuch as a "pure culture" of *P. cheopis* can be obtained, a condition which never obtains in houses, and further by the use of guinea-pig traps an approximate estimate of the number of fleas present at any particular time can be obtained.

Some experiments in the godowns have already been recorded (vol. VII. p. 474), but the following observations amplify these:—

Experiment I. On opening one of the godowns which had been closed for a week, many fleas were seen hopping on the floor. Four guinea-pigs were put into the godown by a man who stayed inside only for a minute or two. When he came out six fleas were caught on his legs. He felt the fleas biting him. Next day the guinea-pigs were examined for fleas and 352 were taken on the four animals.

Experiment II. On another occasion three guinea-pigs which had been in the godown over night were examined for fleas. They yielded 56, 97 and 92 respectively. One of the guinea-pigs after being freed from fleas was returned to the godown for three minutes and then examined. It had picked up 21 fleas in that time. On the legs of the man who went into the godown to fetch this guinea-pig, and who was in for only a few seconds, five fleas were caught.

Experiment III. In another godown on six guinea-pigs, which had been left in overnight, 194 fleas were taken. Three of the guinea-pigs were then inoculated with plague and with the 194 fleas were returned to the godown. Five days later all the inoculated pigs were dead. The man who went in to remove these animals was only in the godown for a few seconds; on coming out three fleas were captured on his legs.

In the above observations as well as in those previously recorded very many fleas were present in the godowns. When the fleas were less numerous man was not so readily attacked, as the following experiments demonstrate.

Experiment IV. Three guinea-pigs had been for some days in a godown known to contain only a few fleas. A man went into the godown and remained in it along with the guinea-pigs for five minutes. On coming out after that period no fleas were found on the man. The guinea-pigs, when examined, gave five fleas. The fleas were now returned to the godown but the guinea-pigs were removed from it. Twenty-four hours later the man again went into the godown for five minutes, this time without any guinea-pigs being present. On his coming out no fleas were found on his legs. A guinea-pig was then put

into the godown for five minutes and then examined for fleas, five being captured on it. The fleas were returned to the godown, which was closed for 24 hours longer. Next day, the fleas having now starved for 48 hours, a man went into the godown for five minutes, no guinea-pigs being present. On this occasion he picked up three fleas, all of which bit him. A guinea-pig put into the godown for five minutes after the man only picked up two fleas.

Experiment V. In another godown, in which from 100 to 200 fleas were judged to be present, two guinea-pigs had been living for some days. A man went into the godown for five minutes, while the guinea-pigs were still present. During this time only one flea came on his legs. The guinea-pigs were then removed and examined, 66 fleas being found on them. The fleas were returned to the godown but not the guinea-pigs. Next day, the fleas having now starved for 24 hours, a man went into the godown for five minutes; eight fleas came upon his legs. A guinea-pig placed in the godown for five minutes after the man had come out trapped 44 fleas. The fleas were returned to the godown and were starved for another period of 24 hours. A man then went into the godown for five minutes and picked up 40 fleas on his legs. A guinea-pig was put into the godown for five minutes after the man came out, and picked up 58 fleas.

These godown experiments confirm the results obtained with fleas kept in glass boxes. We may conclude from all these observations:—

(1) That the guinea-pig is as readily chosen by *P. cheopis* for its host, as its true host, the rat.

(2) That when many rat fleas are present some of them will attack man, even when a rat is available for their food supply.

(3) That when the number of rat fleas is small and when their true host is present they will not attack man.

(4) That when rat fleas are starved they will readily attack all animals, not being particular in their choice of a host.

(5) That rat fleas deprived of food for from 72 to 96 hours attack and feed on man more readily than at other times.

(6) That rat fleas, even when starved, prefer their true host to man.

(7) That rat fleas may be attracted to man, jump on him but take some time to feed on him. Plague infected fleas might in this way be carried from one place to another without infecting the man, but would, when brought near a rat, attack it in preference to man.

III. MODE OF DISPERSAL OF FLEAS.

Fleas being wingless insects and capable of travelling only short distances on the ground, the method of their dispersal from place to place is of interest. This may be effected

- (a) through the host directly in its natural wanderings ;
- (b) with the host when the latter is carried in merchandise :
- (c) by means of merchandise, clothing, grain, etc., the host not being transferred with the fleas.

We shall confine our remarks in this connection to rat fleas.

(a) *Dispersion of fleas by means of the host in its natural wanderings.* We have already drawn attention to the fact that a large part of the life of fleas is spent on the ground, the host being visited more or less temporarily for the purpose of obtaining food, so that rats in their wanderings are constantly picking up and dropping fleas. We took advantage of this fact to keep certain godowns supplied with rat fleas. Rats had access to the roof of the godowns, but were excluded from the interior thereof by a wire netting screen. The fleas on the rats, that moved about or rested upon this screen from time to time, left their host and fell through the wire netting upon the floor of the godown below. Their presence in the godown was readily ascertained by using guinea-pig traps. Some counts of the fleas obtained in this way are given in these reports (vol. vi. p. 453).

Sick rats especially harbour fleas and are therefore more frequently dropping them. On each of four plague-sick rats in Bombay from 80 to 100 fleas were taken (vol. vi. p. 482, Table III). Again, four rats which were found dying from plague in the Punjab village Kasel gave the following number of fleas on each rat, viz. 137, 80, 10 and 35, or an average of 65 fleas per rat. Further, it was a matter of common observation in our godown experiments to note that sick guinea-pigs especially harboured fleas. It will thus be easily understood that a sick rat, crawling with fleas, will probably leave a certain number of fleas behind it, even on passing through a room. Moreover, we have already shown that if this rat was moribund from plague some of these fleas would be plague infected. In other words, a plague-sick rat in its wanderings might well leave a trail of infected fleas behind it.

(b) *Dispersion of fleas with their host when the latter is carried in merchandise, etc.* This method by which fleas may be dispersed requires little more than mentioning. We would, however, like to draw attention to the ease with which rats with their fleas can be transported in certain

kinds of merchandise. We have seen rats dive, as it were, into bags containing bran and disappear, so that the bags could be moved without any evidence of the presence of the rats within. *M. rattus* from its habits is particularly liable to be transported in this way.

(c) *Dispersion of fleas by means of merchandise, grain and clothes in the absence of their host.* From what has been said above it will be apparent that merchandise and grain, which have been visited by rats, may have fleas deposited on them and these fleas may be transferred with these articles to distant places. It is necessary to qualify this statement by pointing out that adult fleas, in the absence of any host to feed on, rapidly die, generally in about five days. However, larvae, since they can feed upon almost any kind of organic rubbish, and pupae, which require no food, could be carried considerable distances in merchandise, *i.e.* for periods as long as one or two months. The larvae and pupae so carried would in course of time develop into adult insects, other circumstances being favourable, but would then require a host to feed upon. In the absence of a suitable host they would perish within a fortnight of the time of their development into the adult or imago state.

During our visits to infected quarters in Bombay City we had many opportunities of noting that we carried away fleas on our persons or on our clothing. These fleas were generally human fleas, but occasionally *P. cheopis* was obtained. Moreover, in a number of experiments in which we caused clothes to be brought to the laboratory from infected houses, we were able to capture not only human but also rat fleas.

Fleas will be more readily carried on the clothes of a person, in that the man who wears the clothes forms an attraction for the insects. A reference to a previous paper (vol. VII. p. 472) and a consideration of the experiments carried out in the godowns, to be detailed later, show how readily and in what large numbers rat fleas may, under certain circumstances, come on to man, and leave us certain that rat fleas must frequently be transported in this way from place to place, especially from plague infected houses, where they are more likely to take to man because of the absence of their true host.

In whatever way fleas are transported, whether in clothing or merchandise, etc., they will select, when carried to their new surroundings, their true host or the next best available animal.

IV. THE COLLECTION AND EXAMINATION OF FLEAS.

The collection of fleas. All are familiar with the great agility of the flea. The nimbleness of the insect, together with its minute size and flattened body, capable of withstanding considerable pressure, helps it to elude its enemies, especially man. This observation, however, chiefly refers to the human flea which progresses by hopping more frequently than many other species. These other species prefer to run rather than hop, and display their agility to the best advantage among the hairs of their host. Practice, however, soon enables one to capture fleas with the unaided fingers. Once caught between a finger and thumb they are readily transferred to a test tube, if into the mouth of the tube a smooth glass funnel is inserted. The fleas dropped into this funnel are unable to obtain a footing on the smooth glass and fall down into the tube.

The use of chloroform. Fleas, however, are more easily captured with the aid of chloroform. A few drops of chloroform are placed on a small pad of absorbent cotton wool. As soon as a flea is seen the pad is placed over it and held there for half a minute or so. The flea becomes anaesthetised and can be readily picked up in the fingers or by means of a moistened brush. This method is most applicable to the capturing of fleas on man and other large animals. Another method can be adopted if the animal from which the fleas are to be taken is of comparatively small size. The animal is placed in a large glass bottle or jar upon the bottom of which a little chloroform has been dropped. The fleas crawl out from among the hairs to fall upon the bottom of the jar or remain entangled among the hairs. As soon as the animal has become completely anaesthetised, it may be removed from the bottle and the fleas can be picked off it. The effect of the chloroform on the animal is a guide to its effect on the fleas. When transferred to a test tube, the fleas rapidly recover from the anaesthesia and can be used for experimental purposes.

The use of a tray. Another method which proved to be very convenient for the collection and handling of fleas, especially in our cage experiments, was to empty the contents of the cage, *i.e.* sand and rubbish, upon a large white tray, which had smooth vertical sides about six inches high and all angles rounded off. If this tray was placed near a window and the sand from a cage spread out in a thin layer upon it, the fleas tended to make their way to the side of the tray most distant from the light. Here the fleas could be readily captured as they unsuccessfully

attempted to climb up the smooth vertical sides of the tray. The sand contained in a cage could thus be effectively examined, those fleas which were not on their host at the time of examination being captured in this way. The method was of particular use when an animal had been dead some time before it was examined. Under these circumstances the fleas had left their host and were to be found in the sand contained in the cage. In our early experiments a fresh animal, generally a guinea-pig, was used to trap the fleas, but the adoption of the tray method avoided the loss of a considerable number of animals, which died of plague when they were used as traps for infected fleas.

Capture of fleas in houses.

1. *The use of animal traps.* While it is a comparatively easy task to collect fleas on an animal which harbours them, it would appear at first sight to be a much more difficult operation to capture them in rooms in which they may be present. This, however, can be readily accomplished by using animal traps. To detect the presence of a particular species of flea in a room or place where it may be present, it is necessary, in order to obtain the best results, to use an animal which, if not the true host of that species, is one which is readily selected by it in preference to other animals. For capturing rat fleas *P. cheopis*, the guinea-pig or man serves as a suitable trap. The former animal should be chosen in preference to the latter, not only because it is more acceptable to *P. cheopis*, but because of the danger to the latter in the presence of plague. We have, however, on unavoidable occasions used man as a trap for *P. cheopis*, taking care that he had been well immunised against plague with a suitable vaccine. The trap animals can either be allowed to move about freely through the room or be confined in suitable cages. In the former case a larger take of fleas will be made, because they are picked up in various parts of the room, while in the latter case only those fleas which have been attracted by the smell in the immediate vicinity of the host and have succeeded in reaching it will be captured. Figures illustrating the advantage of allowing the animals to run about freely will be found in vol. vi. p. 482, Tables I, II, V and VI. As fleas are more or less nocturnal in their habits it is advantageous to leave the animal traps overnight in a room. The animals which had been used to trap the fleas are examined in the way detailed above.

2. *The use of tanglefoot.* The above methods are most applicable for the capturing of fleas which spend some little time on their host, but especially in the case of the human flea, which spends only a short time on its host, another method may be adopted. This method consists in placing an animal in a cage surrounded on all sides by a layer of tanglefoot, a sticky resinous preparation spread on paper. Such a cage is illustrated at page 478 of vol. vi. The fleas, attracted to the animal within the cage, in their endeavours to reach it hop on to the tanglefoot where they remain fixed. They can readily be removed from the tanglefoot with a needle and freed from the sticky material by washing in methylated spirits.

By a series of experiments we determined that a flea was unable to hop a greater distance than $4\frac{1}{2}$ inches and by allowing a layer of at least six inches all round, the animal was afforded complete protection from fleas.

Examination of fleas.

1. *Examination of living fleas.* The examination of living fleas is best accomplished when they are under the influence of chloroform. If a few drops of chloroform are placed on the cotton wool plug of a test-tube, the fleas contained therein soon come under the influence of the drug and remain helpless for from ten to twenty minutes, during which time they can be handled and examined. They should be placed in rows on an ordinary glass slide and examined under the $\frac{1}{4}$ inch objective of an ordinary microscope or under the stereoscopic dissecting microscope of Braus and Drüner. The former instrument is the better one for beginners as the magnification is greater and errors in identification are therefore less likely to be made. Increased experience, however, enables one to identify the majority of fleas either with a low power lens or even with the naked eye. It must be remembered, however, that some species of fleas so closely resemble one another that a complete dissection can alone enable one to make a correct diagnosis (see vol. vii. p. 446).

2. *Examination of dead specimens.* Dead specimens are most accurately identified by dissection. In this examination attention should particularly be directed to the eighth and ninth segments of the abdomen. Various methods have been devised to facilitate these dissections, such as boiling the fleas in caustic soda or potash. We have found, however, that dissections can most easily be made if the dead fleas are allowed to soak in water at a temperature of about

80° F. for from 24 to 48 hours so that they become partly decomposed. The chitin, withstanding as it does putrefaction for a longer period than the soft membranous portions of the body, can be readily separated into segments where these segments were jointed together by membrane.

Microscopical specimens can be readily prepared by boiling the fleas in glycerine and mounting them in that medium, or better in glycerine jelly. More conveniently they may be boiled in alcohol, cleared in cedar wood oil and mounted in Canada balsam.

XXX. THE MECHANISM BY MEANS OF WHICH THE FLEA CLEARS ITSELF OF PLAGUE BACILLI.

FLEAS that have fed on septicaemic blood are capable of conveying infection to fresh animals on which they feed. This capability is associated with the presence of plague bacilli in the stomach, intestine and faeces.

If such infected fleas be kept in captivity, it is found that after a time they are no longer capable of conveying infection; at the same time on dissection no bacilli are found in them.

The question arises, what is the nature of the mechanism by means of which infected fleas rid themselves of bacilli?

We shall show that:

1. A clearing process exists.
2. The clearing process is more active at 90° F. than at lower temperatures.
3. That it is probably of a phagocytic nature.

1. *A clearing process exists.*

The existence of a clearing process was suggested by the fact that after feeding on the same septicaemic blood, the number of bacilli in the stomachs of a batch of fleas is very variable. A few hours after feeding all degrees of infection are found to exist, varying from crowded masses to such small infections, that it is only by continued search that any bacilli are recognisable. Further, in about 50 % of the fleas examined, no bacilli are discoverable by the microscope. Slight variations might be expected to occur but without the aid of some process of clearing it is difficult to account for the often observed fact of some fleas being free from bacilli and others being crowded to excess.

If a number of fleas be fed upon a septicaemic rat and subsequently kept under observation being meanwhile nourished upon healthy animals, the proportion found to be infected steadily diminishes day

by day (see Table I). At the same time a considerable mortality occurs so that the decrease in infected fleas might be due to those harbouring plague bacilli dying more rapidly than those uninfected.

TABLE I. *Showing the percentage of fleas found infected on successive days after an infecting meal.*

Day	Percentage infected	Day	Percentage infected
2	38	6	15
3	37	7	8
4	53	8	16
5	21		

To ascertain whether such was the case, experiments were made with infected and healthy fleas and the mortality compared. The results are given in Table II from which it is evident that there is no greater mortality amongst infected fleas than usually occurs amongst healthy fleas under the same conditions, viz. about 10 % per day.

TABLE II. *Showing the death-rate of fleas.*

On each day the percentage alive is shown.												
Days old	1	2	3	4	5	6	7	8	9	10	11	12
Infected	100	78	60	50	34	30	26	16				
Healthy at 75° F.	100	75	65	58	48	38	27	21				
Healthy at 90° F.	100	72	56	42	44	39	34	28	21	19	11	10

It has been already pointed out that the plague bacilli in infected fleas are always confined to the alimentary tract, and from the experiments just mentioned it appears that the existence of numerous plague organisms in the stomach and gut of fleas does not materially affect the health of the insects.

2. *The clearing process is more active at 90° F. than at lower temperatures.*

In Table II above it is shown that bacilli can be recognised in the stomachs of infected fleas up to at least the eighth day after the infecting meal. The recognition was a microscopical one. Using the same means, but maintaining the fleas at a temperature of 90° F., no bacilli were recognisable after the second day.

Thus:

Day	Percentage infected
2	8
3	0
4	0
5	0

It will be noted here that the proportion found infected on the second day is 8 as compared with 38 at the lower temperature.

When we employ the more delicate test of inoculation (of faeces) into animals for recognising the presence of plague bacilli the same fact becomes evident, but the time is longer.

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
90° F.	+	+	+	+	+	+	-	+	-	-	-	-	-	-	-
75—80° F.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Day	16	17	18	19	20	21	22	23	24	25	26	27	28	29	
90° F.	-	-	-	
75—80° F.	+	+	-	-	+	+	-	-	-	-	-	-	-	-	

In this series of experiments the fleas were infected at the lower temperature, and in the one case maintained at that temperature during the subsequent course of the experiments and in the other removed to the higher temperature immediately after infection. In another series, the infection was carried out at the higher temperature and the fleas subsequently maintained at that temperature:—

Day	1	2	3	4	5	6	7	8	9	10
	+	+	+	-	-	-	-	-	.	.

It is evident that not only is infection less likely to take place at the higher temperature, but that if it does occur, the period during which it lasts is still further curtailed.

A process by means of which the flea rids itself of bacilli, therefore, exists—What is its nature? *A priori* a purely physical clearing process must exist, *i.e.* as the flea continues to feed hour by hour and day by day, and as the bacilli continue to multiply, the number of bacilli present at any time will be dependent on the relative activities of these two processes. If the multiplication can keep pace with the washing out of the intestine by successive meals of fresh blood, then the bacilli will be found in considerable number, and *vice versa*. But in addition to this method another method exists as is evident from the results of experiments detailed above.

3. *This process is probably a phagocytic one.*

The bacilli multiply in the blood of the rat which is taken into the stomach but they are at the same time subjected to all the influences which blood is capable of exerting on bacteria. These influences will be most active immediately after a meal and gradually lessen as the

blood becomes altered by the digestive processes. First in importance amongst such influences we place phagocytosis.

If a well infected flea be fed on fresh blood and after the lapse of some fifteen minutes be killed and a film of the stomach contents stained with Leishman's stain the polynuclear cells are seen to be engorged with plague bacilli.

The effect of increasing the temperature at which the fleas were kept upon the rate at which the insects cleared themselves of plague bacilli is seen on reference to the experiments detailed on p. 262 above. These experiments show that a rise of temperature of about 8 degrees centigrade (75° F. to 90° F.) doubles the rate at which the bacilli disappear, *i.e.* 2·3 times per 10°. Phagocytosis has long been known to be increased by a rise of temperature up to 40° C., and recent observations on the temperature coefficient of phagocytosis by Ledingham have shown that for a rise of 10° C. the velocity is increased about 2·2 times. Ledingham has further shown that temperature operates principally upon the opsonization and only slightly if at all upon the actual process of uptake of bacilli (*Proc. Roy. Soc.* read Feb. 27, 1908).

The process of clearing is also influenced by other conditions which are known to affect phagocytosis.

The presence of bacilli in the stomach of the flea, and the length of time they persist therein, is largely influenced according as the bacilli are subjected to the action of fresh blood immediately after the infecting meal or not; so that the extent of infection is dependent on whether it feeds on healthy blood immediately after its infecting meal, or whether it is starved for a while.

The first series of experiments consisted in infecting fleas, and in one case allowing them to feed on healthy animals immediately after infection and in the other immediately after infection starving the fleas for six hours. In each case the faeces of the fleas were injected into guinea-pigs each day to ascertain the presence or absence of bacilli. The result of this series of experiments is shown in Table III, which shows that twice as many starved fleas had bacilli in their faeces as the fed fleas on the same day.

In the second series of experiments infected fleas were fed for the same time, in one case on animals immunised against plague by large doses of living bacilli and in the other case on animals susceptible to the disease. After feeding for 24 hours on these animals the fleas were transferred to guinea-pigs, which are very susceptible to plague. This experiment was repeated eleven times and from the table (IV) it will

TABLE III. *Table showing the presence (+) or absence (-) of plague bacilli in the faeces of infected fleas on various days after infection for two classes of fleas.*

1. Fleas starved for 6 hours after infection. Total 31 deaths.

Exp.	Days						
	1	2	3	4	5	6	7
1	+	+	+	+	+		
2	-	+	+	-	+	+	+
3	+	-	-	+	+	-	-
4	+	+	+	+	+	+	+
5	-	+	+	-	-	-	-
6	+	+	+	-	-	-	-
7	+	+	+	+	+	-	
8	-	-	+	-	-	-	-
9	-	-	-	-	-	-	-

2. Fleas fed immediately after infection. Total 15 deaths.

1	-	-	-	-			
2	-	-	-	-	-	-	-
3	+	-	+	-	-	-	-
4	+	-	-	-	-	-	+
5	+	+	+	-	-	-	-
6	-	+	+	+	-	-	+
7	-	-	-	-	-	+	+
8	+	+	-	-	-	-	-
9	-	-	-	-	-	-	-

TABLE IV. *Table showing effect of feeding infected fleas, in one case on susceptible animals and in the other on immune animals for 24 hours, on the power of the flea to transmit the disease.*

Experiment A.	Fleas fed on susceptible animals	Experiment B.	Fleas fed on immune animals
1	+	1	+
2	+	2	-
3	-	3	-
4	+	4	+
5	+	5	-
6	+	6	-
7	-	7	-
8	+	8	+
9	+	9	+
10	+	10	-
11	-	11	-

+ means that the fleas subsequently transmitted plague to a guinea-pig.

- means that the fleas failed to subsequently transmit plague to a guinea-pig.

In all cases the fleas were left on the guinea-pigs and the guinea-pigs observed for a month.

be seen that the fleas which had been fed on immunised animals were just half as capable of infecting the guinea-pigs as were the fleas that had fed on the non-immunised susceptible animals.

It appears therefore that a flea starved after the ingestion of septicaemic blood offers the greatest chances of conveying infection, that these chances are diminished if the insect has in the meantime taken a meal of normal blood, and that a meal of the blood of an immunised animal still further diminishes the infectivity of these insects. A probable explanation is afforded by the discovery that the plague bacilli remaining in the stomach are readily ingested by the leucocytes taken in with the second meal and such phagocytosis might be expected to be more pronounced in the case of immune blood.

Should this hypothesis on further investigation prove correct then it follows that the presence of immune rats in epizootic times will tend to limit the advance of the disease; and it will do this in two ways—not only will immune rats not be attacked by plague, but by serving as a source of immune blood to fleas already infected will diminish their power of transmitting the disease to susceptible animals—rats or man. Whether such a process operates in bringing an epidemic to an end we have no means of judging, but from other evidence we believe that the proportion of immune rats does rise at the end of an epizootic, as indeed it might have been supposed to do.

XXXI. ON THE SEASONAL PREVALENCE OF PLAGUE IN INDIA.

INTRODUCTION.

ONE of the most striking feature of plague epidemics is their seasonal prevalence. This feature has been well marked in India since the disease was introduced into Bombay in the autumn of 1896. In places where plague has once become established, the epidemic period, lasting as a rule about 3 months, tends to recur always at the same season of the year. This yearly outbreak, however, does not coincide in point of time in different places in India, but, as we shall see, varies considerably in this respect. Further, it is to be noted that the first epidemic may be at a different season of the year from that which obtains in all subsequent outbreaks.

We propose in this communication to enquire whether the facts of seasonal prevalence are consistent with the view that, as far as epidemics are concerned, the prevalence of plague depends upon the transmission of the plague bacillus from rat to rat and from rat to man by the rat flea. We propose to approach the problem, first by stating briefly the facts of seasonal prevalence as observed in six places in India. These places were specially chosen for the reasons, that they are more or less widely separated from one another, that they differ markedly from one another as regards their climatic conditions, that plague has annually recurred in them for the last 4 to 10 years, and that the yearly outbreak occurs at different seasons in the different places. Secondly, we shall proceed to state what possible factors, having regard to the part played by the rat and the rat flea in the epidemiology of the disease, might influence the rise and fall of the epidemic. Finally we shall discuss in detail each of these factors, bringing forward any experimental evidence which bears on each hypothesis. We do not propose to consider at this time the further problem of how the intervals between the acute epidemics are filled in, as the investigations on this question are not complete.

I. FACTS OF SEASONAL PREVALENCE OF PLAGUE IN
SIX PLACES IN INDIA.

1. *Bombay City.*

Bombay City, with a population of about 1,000,000 inhabitants, is built on an island on the west coast of the peninsula. The climate on the whole is hot and damp. The daily mean temperature, considered over the whole year, is between 70° and 80° F., the mean diurnal range being only 12·5° F. It is subject to the south-west monsoon which blows from May to October, and during which period, especially in June, July and August, there is a heavy precipitation of rain. During the rest of the year the north-east monsoon prevails, there being at this season little or no rain.

Plague was recognised in Bombay in the latter half of September 1896. Up to the end of 1896 it did not assume any formidable proportions, the first real epidemic being in the spring of 1897. Since then the seasonal prevalence of plague in Bombay has been well marked and has shown little or no variation (Chart I). A study of the curve¹ will show that each year the epidemic begins in January, gradually rises until it reaches its maximum in March, then rapidly declines until by the middle of May the plague mortality has returned to what it was before the epidemic. Different years show a slight variation from this general scheme. For example, in 1900 the epidemic began a little earlier than usual, that is to say in December 1899, and lasted perhaps a little longer, while in 1906 it did not begin till February and was a little later in subsiding, namely the end of May. This latter phenomenon was also observed in 1905. Further, a study of the curve will show that in several years, especially in 1898 and 1901, there was a slight recrudescence in the months of August and September, which recrudescence, however, never reached the same proportions as the epidemics in the spring. We can, therefore, state that the seasonal prevalence of plague in Bombay is from January to April and that the factors which determine the epidemic must be most in evidence at this time. While this is so, as there is often a tendency to recrudescence during August to December,

¹ The method on which this and all subsequent curves are constructed was as follows. The bimonthly plague mortality for the period over which the curve extends was taken, and a mean of these figures calculated. Then the percentage above or below this mean for each bimonthly figure was determined and the curve constructed on these percentages. In this way the relative severity of the different epidemics in the same place can be seen at a glance.

the conditions at this season must be more favourable than in the latter half of May, June and July, when the plague mortality is always at its lowest.

2. *Poona, City and Cantonments.*

Poona is only 80 miles distant from Bombay. It has however different climatic conditions and a different seasonal prevalence of plague. Poona is situated on the plateau of the Deccan about 40 miles inland from the summit of the Western Ghats, and about 2000 feet above sea level. The daily mean temperature for the year is 70° to 80° F., while the diurnal range is on an average 22.5° F. Poona is subject to the south-west monsoon during the months of May to October. As, however, it lies some distance inland the rainfall is much less than on the coast, the greatest precipitation taking place on the Ghats. During the months of March, April, May and June, Poona is hot but dry, the daily mean temperature being between 83° F. and 90° F. In July owing to the cool south-west breezes there is a marked fall which is sustained during the autumn months, the mean daily temperature during this season being between 75° and 80° F. In the winter months Poona enjoys a delightful climate, the mean daily temperature being about 70° F. and the diurnal range about 30° F. A study of the curve (Chart II) will show that plague was first introduced into Poona in the beginning of 1897. In February and March of that year there was a distinct but comparatively slight epidemic which reached its height at the end of March and rapidly subsided during April. This is the only outbreak which Poona has had at this season of the year. The other epidemics all occurred between August and March and can be divided roughly into two groups, namely:

(a) A group, in which the plague mortality begins to rise in August or September, reaches its height about the beginning of October and then comes down rapidly afterwards. We may call this group, into which fall the epidemics of 1897, 1900 and 1906, 'early epidemics.' We can also include in this category the epidemic of 1899, which however, as reference to the curve will show, began as early as March, but remained latent during the hot weather, rising during August to an abnormal height.

(b) A second group, in which the epidemic does not begin till October or even December, as in 1902—1903, reaches its maximum in December or January and then falls till the mortality becomes normal in February or March. This group we may call 'late epidemics.' The

epidemics of 1901—1902, 1902—1903, 1903—1904, and 1904—1905 were of this description.

It does not appear that there is any great difference between the early and late epidemics as regards their severity, although, if anything, with the exception of the late epidemic of 1902—1903, the early ones have been more severe. It is noteworthy that there was no epidemic in 1898, nor again in the autumn or winter of 1905. On the whole, therefore, a study of the Poona epidemics leads us to conclude that while the most favourable time seems to be the autumn, namely, August to November, nevertheless the conditions must still be favourable during December to March. Further, it appears that from March to the beginning of July plague cannot exist in epidemic form.

3. *Nagpur City.*

Nagpur is a fairly large city situated in the centre of the Central Provinces, of which it is the capital. The climate is not unlike that of Poona, except that the mean temperature is higher both in the hot weather and in the rainy season. The hot weather begins in March, and lasts into June, the mean temperature being then between 85° and 95° F. In July, August and September, the mean temperature is a little over 80° F.; while in the cold weather during November, December, January and February it falls to between 70° and 75° F. The rainfall is almost all confined to the months of June to September and during this period there is an average fall of about 40 to 60 inches.

The curve (Chart III) shows that plague was first introduced in the cold weather of 1902—1903. During January and February of 1903 there was a rapid rise, the mortality reaching its highest point in the beginning of March. From this date it rapidly fell till by the end of April the plague mortality was practically nil. The next epidemic in Nagpur began in October 1903, reached its height in December and January and then rapidly fell till by the middle of March it had practically subsided. The remaining part of 1904 and practically the whole of 1905 were free from plague. The last epidemic began in December 1905, rose rapidly during January and February 1906, and reached its summit in the beginning of March. From this time it rapidly declined till it had practically subsided by the middle of April.

It will therefore be seen that Nagpur has suffered from three epidemics, two during the months of January—March, and the third

between October and February. Finally we may take it that in Nagpur the conditions on which seasonal prevalence depends are most favourable during the months from November to March. Epidemic plague has been unknown between April and September inclusive.

4. *Belgaum City.*

Belgaum is a city situated in the southern Maratha country, that is to say, in the southern extremity of the Bombay Presidency. It lies on a plateau about 2500 feet above sea level and 75 miles inland from the west coast of India. The climate of Belgaum is one of the most equable in India. During the greater part of the year, namely, from June to February the mean daily temperature is between 70° and 75° F., the average diurnal range being about 20° F. During the hot season, March to May, the mean daily temperature never rises very high, ranging about 80° F. The average annual rainfall is about 40 inches, most of which falls between June and October. At this season the humidity is at its maximum.

As regards the plague epidemics which have visited this city, a study of the curve (Chart IV) will show that the disease was first introduced in October 1897. This epidemic, a comparatively slight one, lasted throughout nearly the whole of the winter months, reaching its maximum in January 1898, and disappearing in the spring of that year. All subsequent epidemics which have appeared in Belgaum have, with the exception of that of 1899, commenced in July or August, reached their maximum in October and disappeared towards the beginning of the new year. The epidemic of 1899 began in May, reached its height in August and subsided by October. It was, in short, two months earlier than usual. It is evident, therefore, that the epidemic season of plague in Belgaum is August—December, but that an epidemic may start earlier and come to an end before the time of year at which the usual outbreaks cease. Another interesting observation which is obtained from a study of the curve is, that since the epidemic of 1898, which was very severe, all subsequent epidemics, with the exception of that of 1902, have decreased in severity year by year until 1906, in which year there was practically no plague at all. It will be interesting to watch and see if there is any recrudescence in 1907.

5. *Lahore City.*

Lahore, the largest city and capital of the Punjab, is situated nearly in the centre of the plains of this Northern Province. It is about

700 feet above the sea level. Owing to its geographical situation Lahore is removed from the tract of the south-west monsoon and in fact receives comparatively little rain during the year. The climate of Lahore shows marked differences at different seasons of the year. From November to March the mean temperature is below 70° F., falling in January to about 54° F. During the rest of the year the mean temperature is above 70° F., in May, June, July and August ranging between 85° and 95° F. The heat during these months is intense. The diurnal range of temperature is extensive, being on an average 27·5° F., while in April and May it is 32·5° F., and in October and November as much as 35° F. The rainfall during the year is small, between 20 and 25 inches, the chief fall taking place in the months of July, August and September. There are also winter rains during January and February, much less in amount however than the autumn fall.

A study of the plague curve (Chart V) will show that the disease was first introduced into Lahore in the early part of 1902. There was no well-marked epidemic during the following spring, a slight outburst coming to an end with the advent of the hot weather in May. During the winter of 1902—1903 there was a slight recrudescence; but the first serious epidemic took place in the spring of 1903. This outbreak, beginning in March, reached its height early in May, after which date there was a rapid decline, until by the end of June the mortality was normal. The second epidemic, the most severe from which Lahore has suffered, began in March 1904, reached its maximum early in May, and then fell rapidly, until it had practically disappeared by the end of June. The third and fourth epidemics, namely those of 1905 and 1906, occurred at the same season of the year as the first and second. Further, during the cold weather of 1904—1905 and of 1905—1906 there were slight recrudescences, which, however, never reached the proportion of epidemics. Finally, we have to note that in October—November 1906 there was a marked increase in the plague mortality, which by the end of December was still on the upward tendency. Here evidently the winter recrudescence was assuming the proportions of a spring epidemic.

Viewing the epidemics in Lahore as a whole we conclude that March—May are the three months in which plague shows its most marked seasonal increase, that there is a tendency to recrudescence during the winter months and that during the hottest months of June—September the disease is never present in epidemic form.

6. Rawalpindi City and Cantonments.

Rawalpindi, a large native city with an extensive military cantonment adjoining, is situated in the north of the Punjab at the base of the Himalayas. The climate is not unlike that of Lahore but, as Rawalpindi is considerably further north and lies 1700 feet above sea level, the hot weather is not so severe nor so prolonged as in the former place. Further, situated as it is at the base of the Himalayas it receives more rain than Lahore. The daily range of temperature is considerable, namely, between 20° and 30° F. In the hot weather, namely, May, June, July and August, the mean temperature ranges between 80° and 92° F. The cold weather has an earlier onset and is more severe than at Lahore, the mean temperature at the coldest period falling to below 50° F. The rainfall averages 30 to 40 inches per annum and while the principal fall is in July, August and September, the winter rains during January to April are not inconsiderable, namely, about 8 inches.

Plague first appeared in Rawalpindi (*vide* Chart VI) in the hot weather, namely June of 1903. It did not, however, assume epidemic magnitude till two months later. By September it had grown into a severe outbreak. This epidemic reached its maximum at the beginning of October, from which date it fell rapidly, until by the end of November it had disappeared. During the next 18 months, while there was no serious epidemic, there were several small recrudescences, the most marked in June 1905. This latter outbreak remained latent during the hot weather, bursting out into a small epidemic in the autumn. It reached its height early in November and by the end of the year had entirely disappeared. The plague mortality began to rise again in May 1906; did not increase during the hot weather, but rose to become a serious epidemic in the autumn. This outbreak reached its height towards the end of October and by the end of December had practically disappeared.

A study of the Rawalpindi curve (Chart VI) as a whole shows us that evidently the most suitable season for plague epidemics is the autumn, September to November, but that slight recrudescences may occur practically at any season of the year. It is especially noteworthy that in every year the disease has been present in the city in the spring, but on the three occasions on which an epidemic followed, this did not occur till the autumn.

II. POSSIBLE FACTORS WHICH INFLUENCE THE SEASONAL
PREVALENCE OF PLAGUE.

We have now to pass on to a statement of the factors, which on the basis of the rat-flea-man theory might influence this seasonal prevalence. We can classify these factors as follows:—

A. *Climatic conditions, especially temperature, rainfall and humidity.*

When we came to consider in what way temperature might act it appeared to us that it might be by a direct influence on the transmission of the bacillus from animal to animal by the flea, that is to say, on the conditions to which the bacillus is subjected in the stomach of the flea, or by a direct influence on the disease in the rat, especially as regards the degree of septicaemia. Further, it is readily conceivable that climatic conditions might affect the seasonal prevalence of plague in so far as they influenced the life history and habits of the rat or of the flea, or the habits of man.

B. *Variations in the virulence of the bacillus.*

It has been suggested by some observers that the virulence of the plague bacillus is diminished by passage through the rat. If such a diminution occurred in nature or if there was any variation in virulence due to other causes, it is possible that such variation might influence the seasonal prevalence of plague, inasmuch as a bacillus of diminished virulence would tend to cause a localised, rather than a general infection, which latter condition is essential to enable the flea to become infected.

C. *Variation in the total number of rats and variation in the proportion of immune to susceptible rats.*

It is conceivable that the seasonal prevalence of plague might be dependant upon a variation in the total rat population or upon a variation in the proportion of immune to susceptible rats. The effect of a plague epizootic on the rat population must be to diminish, for the time being, the total number of rats and to leave a greater number of rats which as a result of recovery from mild attacks of plague are more or less immune to the disease. It is possible that an epizootic might be influenced in the direction of being brought to an end by these causes. Further, if there was a definite breeding season of rats, as a result of

which the rat population was at one season of the year increased by a large number of young non-immune animals, we would have the conditions, as far as the rat population is concerned, suitable for the recrudescence of the epizootic. It is evident, therefore, that we shall have to consider the above as possible factors influencing the seasonal prevalence of the disease.

D. *Variation in the number of fleas.*

If we accept the flea as the only material means of transmission of the plague bacillus from animal to animal, there are *a priori* grounds for concluding that the severity of an epidemic would be directly influenced by the numbers of fleas present. With a small number of fleas the chances of infection would be less, few cases would occur and the rate of progress of the disease would be decreased. A great flea prevalence would have the opposite effect, namely, a large number of cases in a short interval of time, in other words an epidemic outbreak of the disease. We shall, therefore, have to enquire into what evidence we possess as to there being a marked seasonal prevalence of the rat flea.

III. ANALYSIS OF THE INFLUENCE OF TEMPERATURE IN SIX PLAGUE INFECTED LOCALITIES.

We have already considered in a general way the relation of plague epidemics to different seasons of the year as observed in six widely separated districts in India. We have seen that outbreaks may occur at practically any season. As, however, the climatic conditions, especially temperature, show marked variations in these six places at any one season we have added to the plague mortality curves a curve of the daily mean temperature and a curve representing the degree of humidity. The temperature curve was constructed as follows: from the daily maximum and minimum temperatures, the daily mean temperature was calculated. In the case of Bombay, however, the mean temperature was determined from hourly records. The average daily mean temperature for each half month was then calculated, and the curves plotted out from these figures. The humidity curves were made in the same way, saturation being 100°.

A study of the plague mortality and temperature curve shows the following facts.

Bombay. (Chart I.)

The plague epidemic begins each year when the mean temperature is at its lowest point, namely, between 72° and 75° F.; it rises gradually as the temperature rises and assumes its maximum proportion when the mean temperature is about 78° — 80° F.; very soon, however, after the mean temperature rises above this latter figure, the plague mortality begins to decline, the decline, as the temperature rises, being very rapid. While in the non-epidemic season, namely, May to October, the mean temperature is always above 80° F., there is observed to be a slight fall of temperature in August and September, when a recrudescence is liable to occur. It is noteworthy that the epidemics of 1897 and 1900, which began earlier and which, having reached their height, began to decline before the mean temperature passed 80° F. were of longer duration and declined more gradually than was the case with the other epidemics. These data, therefore, would seem to point to the conclusion that, while a high mean temperature, namely 80° — 85° F., may have an influence in limiting plague epidemics, the factor of temperature is not the only factor which is concerned in the seasonal prevalence of the disease.

Poona. (Chart II.)

It will be remembered that plague was introduced into Poona early in 1897 and that the first epidemic was a slight one, coming to an abrupt end during April. A reference to the temperature curve will show that the mean temperature was gradually rising during the rise of the epidemic and stood at 81° F. when the epidemic was at its height. As the temperature rose above this figure the plague mortality rapidly became less. A possible explanation, therefore, of the cutting short of this outbreak is that the mean temperature became too high before the epidemic had gathered its full strength.

We have also seen that the usual epidemics in Poona may be divided roughly into two groups:

(a) a group in which the outbreak occurs between August and November and (b) a group in which the epidemic season is roughly November—February.

The period from the middle of March to June inclusive has been always free from epidemic plague. Now, when we co-relate these data with the temperature curve, we find that the daily mean temperature during the period July to middle of March is never above 80° F. and that during the period middle of March to June the mean temperature

is always above 80° F. reaching nearly 90° F. in April and May. While the epidemics of both groups occur when the mean temperature is below 80° F., ranging between 66° and 78° F., they, however, decline and come to an end while the temperature still remains low, in fact, as we shall see, when the temperature would appear to be most suitable. Another factor, or factors, therefore, must be in operation in determining the decline of these epidemics, a conclusion which we have already reached from the study of the Bombay curves.

The epidemic of 1899 in Poona deserves especial notice. A reference to the chart will show that the plague mortality in this year began to rise in February, when the daily mean temperature was about 75° F.; that with a rising mean temperature it went on increasing till March, when it seemed to receive a check, the mean temperature being now above 80° F. In April, May and June, when the mean temperature remained high, the plague mortality remained low. The epidemic, however, burst out again in July with excessive severity, when the mean daily temperature fell to about 76° F. It would appear, therefore, that this epidemic, beginning when the temperature was suitable, was held in check during the hot months, when the mean daily temperature was above 80° F., until in July, when the temperature fell on the advent of the south-west monsoon, it burst out in full force.

Nagpur. (Chart III.)

As we have already seen, there have been three plague outbreaks in Nagpur, two during the months of January to March and the third between October and February, that is to say, the period from October to March is a season when the conditions are favourable for epidemic plague. A reference to the temperature curve shows that during this period the daily mean temperature is below 80° F., while during the non-plague season, April to September, the mean temperature is above 80° F., reaching as high as 90° F. or above during April, May and June. While this is so, a study of the chart will show that a high mean temperature could not have been the factor which brought to an end the epidemic of the winter 1903—1904. The plague mortality in this outbreak came down rapidly in February, when the mean temperature was between 70° and 76° F. On the other hand, in the case of the other two epidemics the factor of temperature might have been instrumental in causing their rapid decline. Both of these outbreaks began in January, when the daily mean temperature was but a little above 70° F., increased during February, when the daily mean temperature was below 80° F.,

reached their height early in March and fell rapidly during the latter part of March and April, after the daily mean temperature had passed above 80° F. and was still rising. It is noteworthy that these two outbreaks, which seemed to be thus cut short by the temperature, while severe were of shorter duration and declined more rapidly than the third outbreak, which, as we have seen, came to an end irrespective of any rise in temperature.

Belgaum. (Chart IV.)

We have already seen that Belgaum enjoys a most equable climate, the daily mean temperature for the year being between 70° and 80° F. A study of the temperature curve will show that only during two months of the year, April and May, does the mean temperature rise above 80° F. and even then it is never above 83° F. It is evident, therefore, that we could not expect to find the influence of very high temperatures on the seasonal prevalence of plague in Belgaum and such an expectation is borne out by a study of the chart.

The plague season in Belgaum is August to December, when the daily mean temperature is between 70° F. and 75° F. and, as a study of the chart will show, all the epidemics have declined when the temperature was still about this level. While there is the general relationship between the plague epidemics and the mean temperature, namely, that the epidemics occur when the temperature is lowest, the influence of the higher mean temperature is not apparent.

Lahore. (Chart V.)

The epidemic season of plague in Lahore is during the spring, namely, March to May, the four severe outbreaks which have occurred having been at this time of year. When we come to co-relate the daily mean temperature with the plague mortality we find that in March the mean temperature, when the epidemic begins, is about 70° F.; that during April, when the plague mortality is increasing, the mean temperature is rapidly rising and that in May when the epidemic reaches its maximum the daily mean temperature is between 85° and 90° F. The fall of the epidemic takes place abruptly as the mean temperature is still rising. At first sight it would appear that the mean temperature, namely 80° to 85° F., which seemed in Bombay and Nagpur to influence the epidemics in the direction of bringing them to a close, had failed to act in the case of Lahore, and

that it was only when the mean temperature had reached above 90° F. that the plague mortality began to decline. We have, however, to draw attention to two facts which have a bearing on this question. First, a study of the temperature curve will show that the rise of temperature during the spring and early summer in Lahore is much more rapid than in the places we have already considered. As we can imagine that temperature would take some time to act, its influence in Lahore would not be felt until the mean had risen higher than in those places where the onset of the hot weather was more gradual. Secondly, in Lahore, as we have already pointed out, the diurnal range of temperature is very much greater than in Bombay, Poona or Nagpur, this range being in April and May as much as 30° to 35° F., so that there would be a considerable number of hours during which the temperature would be far below the mean. Whether or not the high temperature in May is the cause of bringing the epidemics in Lahore to an end, a study of the chart shows us, that in the hot months of the year which follow May, namely, June to September, when the daily mean temperature is well above 80° F., plague is never epidemic. Lastly, during the winter months, November to February, when the daily mean temperature is low, namely, between 50° and 70° there have been several recrudescences which, with the exception of the one in the end of 1906, did not reach epidemic magnitude. It is to be noted, however, that the onset of the cold weather was delayed on this occasion. This observation raises the question as to whether or not a low mean temperature, such as 50° F., has any influence on plague epidemics.

Rawalpindi. (Chart VI.)

We have already seen that the epidemic season of plague in Rawalpindi is September to November. In September, that is to say towards the end of the hot weather, the daily mean temperature is declining, being then about 80° F. During October and November, when the epidemics are at their height, the mean temperature is still falling, being now below 80° F. The outbreaks have always come to an end in December when the mean temperature is low, namely, between 50° and 60° F.

When we study the chart more carefully we find that each year plague has been present in Rawalpindi in the spring or early summer, but has not assumed epidemic magnitude until the autumn, when the mean temperature had fallen nearly to or below 80° F. There has

never been a severe outbreak during May to August, when the mean temperature is highest. Further, it is also seen from the chart that plague has never been epidemic in Rawalpindi during the coldest months, namely, December—February, when the daily mean temperature falls to about 50° F.

Before passing on to consider the experimental data bearing on the temperature problem, we may sum up the results of our analysis of the relationship between the seasonal prevalence of plague and the daily mean temperature as studied in these six places in India as follows.

1. *It appears that plague cannot exist in epidemic form in any of these places when the daily mean temperature is as high as 85° F.*

2. *If the outbreak is increasing in magnitude while the daily mean temperature is also rising, as soon as or very shortly after this latter has reached 85° F. or a little less, the plague epidemic receives a check and rapidly declines.*

3. *While this is so, it would appear that epidemics may come to an end when the temperature is most suitable. Another factor or factors must, therefore, be in operation in these instances.*

4. *A low daily mean temperature, such as 50° F., may be a factor in limiting plague outbreaks.*

IV. EXPERIMENTAL DATA AS TO THE INFLUENCE OF TEMPERATURE ON THE TRANSMISSION OF PLAGUE BY FLEAS.

The experimental data were got from observations made in the course of the godown experiments and from several series of observations made in the laboratory at different seasons of the year at room temperature and in specially constructed chambers in which the temperature could be regulated.

(a) *Observations in the go-downs.*

In previous papers (vol. vi. p. 450; vol. vii. p. 421) we have detailed a large number of experiments which were carried out in specially constructed cabins or godowns. We shall now refer only to the points which bear on the present problem. It is to be remembered that we have already proved that the rat flea alone was the transmitting agent of the infection in the epizootics which occurred in these godowns.

Table I contains a summary of the experiments which have been carried out in godowns 1 and 2 (*i.e.* non-flea proof) from June 1905 to

March 1907. These experiments may be divided into three groups according to the season of the year at which they were made.

1. Group 1 is made up of experiments 8, 9 and 10. The experiments of this group were carried out in June and July, the off-plague season in Bombay. A reference to the table will show that no marked epizootic developed in any of these experiments. In one instance no guinea-pigs, in another 2 guinea-pigs, and in the third four out of 50 guinea-pigs contracted the disease.

2. Group 2 is made up of experiments 5, 6 and 7, which were carried out during the season between August and November. In this season plague is not epidemic in Bombay but slight recrudescences have been observed on several occasions. It will be seen from the table, that in each of the experiments of this group an epizootic broke out, but that it came to an end before all the animals were infected. Further, a considerable number of days elapsed between the death of the first and last animal, *i.e.* the epizootic was slow in progress.

3. Group 3 is made up of experiments 1, 2, 3 and 4. These observations took place in the plague epidemic season in Bombay. In these experiments all the guinea-pigs died of plague with the exception of one which developed the disease but recovered. Further, in experiments 3, 9 and 10 the epizootic was extremely rapid, killing off 25 animals in from 3 to 9 days.

As we were unable to discover any variation in the virulence of the bacillus obtained from rats in Bombay at different times of the year, the two factors which suggest themselves as determining the differences between the results of the experiments in these three groups are prevalence of fleas and climatic conditions, such as temperature. It is impossible accurately to appraise the relative importance of these two factors. In the experiments of group 1, all of which failed, fleas were not present in very large numbers, but the mean temperature was well above 80° F. In the experiments of group 2, fleas were abundant and the temperature was still above 80° F., although somewhat lower than during the experiments of group 1. In this group the epizootics were of slow progress and came to an end before all the animals had died. It would appear then that the most probable explanation of the limiting of these epizootics is the high temperature which then obtained. In the experiments of group 3, specially 3, 9 and 10, fleas were abundant and the temperature was well below 80° F., between 70° and 75° F. The epizootics in these instances were severe and ran a rapid course.

TABLE I.

No. of experiment	Season of year	Flea census	Daily average mean temperature	No. of guinea-pigs exposed to infection	No. of guinea-pigs which died of plague	Interval between death of first and last guinea-pig	Remarks
1	January 1907	107 on 3 guinea-pigs	72.8° F.	25	25	9 days	Plague epizootic rising.
2	December 1905	115 on last 5 guinea-pigs	75° F.	26	26	7 "	Plague epizootic in Bombay gaining strength.
3	Nov.—Dec. 1905	400 on last 2 guinea-pigs	78° F.	49	49	17 "	Plague epizootic in Bombay beginning.
4	March 1907	1246 on 2 guinea-pigs	78° F.	25	24*	3 " 16 on 1 day	Plague epizootic at its height.
5	Oct.—Nov. 1906	546 on 4 guinea-pigs	80.6° F.	25	23	18 days	Plague epizootic about to begin.
6	August 1906	126 on 25 guinea-pigs	81.1° F.	25	24	21 "	Season when plague shows tendency to recrudescence.
7	Sept.—Oct. 1906	101 on the last 2 guinea-pigs	81.8° F.	25	23	16 "	Ditto.
8	July 1906	50 on 25 guinea-pigs	82.3° F.	25	2	1 day	Plague epizootic in Bombay at its lowest.
9	June—July 1905	49 on 3 guinea-pigs	82.5° F.	50	4	11 days	Ditto.
10	June 1906	39 on 5 guinea-pigs	Minimum never below 80.5° F. and often above 82° F.	25	0	—	Ditto.

* The guinea-pig which did not die was noticed to be ill at the same time as the others. It, however, gradually got better. Three days after the death of the last animal it was killed with chloroform and was proved to be plague infected on bacteriological examination. It would evidently have recovered from the disease.

(b) Experimental observations in cages in the laboratory.

We have made several series of experiments which had as their object the transmission of plague from animal to animal by means of fleas, and in which all the conditions, except the temperature, were kept constant. The same number of fleas, which were always taken from plague infected rats which had numerous plague bacilli in their blood, was used for each experiment. For the general technique of these observations we would refer our readers to the first report (vol. v. p. 446).

These observations may be divided into four groups as follows :

1. *Experiments made at different seasons of the year at room temperature.*

Table II contains the details of these observations. From this table it is seen that both in the case of wild Bombay rats and in the case of guinea-pigs very many more successful transmissions were got in the plague season than in the off-season. It is to be remembered that the same number of fleas were used for each experiment and that only those fleas which had fed on rats, the blood of which contained abundant

TABLE II.

Animals	Season of year	Mean daily temperature	No. of experiments completed	No. of successful transmissions	P.c. of successful transmissions
1. Bombay wild rats	Jan.—March 1906	73—78° F.	29	16	55
	May—June 1906	82·5—85° F.	20	3	15
2. Guinea-pigs	March—April 1906	75—80° F.	14	13	93
	May—June	82·5—85° F.	22	3	13

plague bacilli, were used. It would appear, therefore, that the most likely explanation of the diminution in the number of successful transmissions which was observed in May and June is the high mean temperature which then obtained. In the case of the Bombay rats this diminution might be partially accounted for by the presence of a greater number of immune rats at the end of the plague season, but this cannot apply to the experiments in which guinea-pigs were used. The guinea-pigs were all young animals which had never been used before for experimental purposes.

2. *Experiments made in the hot weather, i.e. in the non-plague season, simultaneously at room temperature and in a specially constructed cool chamber in which the temperature could be regulated.*

These experiments were carried out during the months of July and August, 1906. At this season of the year plague in both rats and men occurs in Bombay, but not in epidemic proportions, though in several years there has been observed a slight recrudescence beginning in August. The experiments were made simultaneously at room temperature (daily mean between 80°—83° F.) and in a cool room. This room was cooled by a carbon dioxide refrigerating apparatus, and was kept at a constant temperature of about 70° F. As the room had to be kept tightly closed the carbonic acid expired by the animals was absorbed by a layer of lime spread on the floor, which also served the purpose of keeping the room comparatively dry. Exactly the same technique was used in the experiments in which the animals were kept in the cool room, as in those in which they were kept at room temperature. The only occasion on which the fleas were removed from the cool room was at the time when they were being transferred from the inoculated animal which had died of plague to the healthy animal. This operation only lasted for about 15 minutes. Working in this way we have completed three series of experiments, using (1) Bombay rats, (2) ship rats and (3) guinea-pigs, as the healthy animals to which the fleas were transferred. The results of these three series are detailed in Table III.

TABLE III.

July—August, 1906.

Animals	Conditions of experiment	Daily mean temperature	No. of experiments completed	No. of successful transmissions	P.c. of successful transmissions
1. Bombay rats	Room temp.	80—83° F.	29	5	17
	Cool room	70° F.	46	13	28
2. Ship rats	Room temp.	80—83° F.	25	8	32
	Cool room	70° F.	27	18	66
3. Guinea-pigs	Room temp.	80—83° F.	8	4	50
	Cool room	70° F.	15	15	100

From this table it is seen that the same result was got in each series, namely, the proportion of successful transmissions at 70° F. was about twice that at 81·5° F. The only factor which could be responsible for this difference appears to us to be the higher temperature at which the experiments outside the cool room were carried out.

3. *Experiments made in the cool weather, i.e. in the plague season, simultaneously at room temperature and in a specially constructed hot chamber, in which the temperature could be regulated.*

This series of experiments was the reverse of the last series. It was carried out during the months of January and February 1907, at a time when both the epizootic and epidemic were steadily rising in Bombay. The experiments were made simultaneously at room temperature, the daily mean temperature being about 75° F., and in a hot chamber which was kept between 85° and 90° F. The same technique was used as was employed in the previous series.

TABLE IV.

January—February, 1907.

Animals	Conditions of experiment	Daily mean temperature	No. of experiments completed	No. of successful transmissions	P.c. of successful transmissions
Guinea-pigs	Room temp.	75° F.	25	21	84
	Hot room	85—90° F.	25	8	32

The results of this series, in which guinea-pigs alone were used, are detailed in Table IV. From this table it is seen that while at room temperature 84 p.c. of the experiments were successful, at the higher temperature of 85° to 90° F. only 32 p.c. gave a successful result. In this series, also, it would appear that the higher mean temperature had hindered the transmission of the disease by the flea.

When we consider the results of these three series of experiments we feel justified in coming to the conclusion that a high mean temperature, namely, above 90° F., has a direct hindering influence on the transmission of plague from animal to animal by means of fleas. The higher this mean temperature the greater is the influence felt. The experimental evidence, both in the godowns and in the laboratory, is therefore in harmony with the facts which were observed concerning the seasonal prevalence of plague in six different places in India.

When we come to consider in what way the higher temperature exerts its influence, we cannot offer an altogether adequate explanation in the present state of our knowledge. There is no doubt that the action must be in the direction of influencing the conditions under which the bacilli are living in the stomach of the flea.

In a previous paper (vol. vii. p. 402) we have compared the fate of the plague bacillus in the stomach of the flea in two series of experiments, one of which was carried out in the cool weather of the

plague epidemic season and the other in the hot weather of the non-epidemic season.

TABLE V.

Season when experiment was carried out	P.c. of fleas with plague bacilli in stomach between 2nd and 6th day after they had imbibed septicaemic blood	No. of days from time of imbibing septic- aemic blood during which plague bacilli were found in stomach of fleas	No. of days from time of imbibing septicaemic blood during which fleas remained infective for guinea-pigs
Cold weather: plague epidemic season }	31.2	20	15
Hot weather: non- epidemic season }	5.2	7	7

Table V contains a summary of these two series of observations. A study of this table shows us that during the hot weather fewer fleas were found infected, and that they lost their power of infecting animals much sooner than in the cold weather. In the hot weather the bacilli disappeared from the stomach of the flea much more quickly than in the cold weather.

Further two series of experiments were done in January and February 1907, one at room temperature, namely 75° F., and the other in the hot room at about 90° F. In these series we tested the duration of the infectivity of the faeces of the fleas. A large number of fleas, caught on the animals when they were removed from the cage each day, were placed in two or three test tubes and left there for about 2 hours. They were then removed and returned to the cage. The tubes were now washed out with a small quantity of sterile normal salt solution, which was forthwith injected subcutaneously into a guinea-pig. This operation was performed each day.

We found that in the case of the fleas kept at room temperature (75° F.) the faeces infected up to the 21st day, and in the case of the experiment done at 90° F. only for 3 days. It would appear, therefore, that the higher temperature acts in the direction of clearing the bacilli out of the stomach of the flea. On what this clearing action depends is a problem which is dealt with in a separate paper (this vol., p. 260).

4. *Experiments made at low temperature.*

From a study of epidemic plague in relation to temperature in Lahore and in Rawalpindi it appeared that a low mean temperature such as 50° F. might be a factor in limiting plague outbreaks. We have investigated this point experimentally. Three series of flea transmission experiments were carried out with guinea-pigs in a cool chamber at

40° F., 50° F. and at 60° F. respectively. The technique of these experiments was the same as has already been described.

The results were as follows:—

At 40° F. 10 experiments were carried out: three of the guinea-pigs soon died of cold, the other seven all succumbed to plague.

At 50° F. 10 experiments were done: the 10 guinea-pigs all died of plague.

At 60° F. 10 experiments were done: all 10 guinea-pigs died of plague.

It would appear, then, that low temperatures, under the circumstances of these experiments, do not hinder the transmission of the disease by fleas. It is to be remembered, however, that only those fleas which were taken from rats which had died with abundant plague bacilli in their blood, were used.

TABLE VI.

		Mean temp. at which rats were kept	No. of rats which died of plague	Average no. of days between inoculation and death of rat	P.c. of rats in which plague bacilli were found in blood	P.c. of rats in which abundant plague bacilli were found in blood	P.c. of rats in which no plague bacilli were found in blood
1. Series 1	(a)	82.5° F. Room temp.	81	3.0	69	33	31
	(b)	40° F. Cold room	72	2.1	10	3	90
2. Series 2	(a)	80.6° F. Room temp.	60	3.1	70	33	30
	(b)	50° F. Cold room	107	2.4	19.5	7.5	80.5
3. Series 3	(a)	76.8° F. Room temp.	19	3.2	79	58	21
	(b)	60° F. Cold room	36	2.7	41	25	59
4. Series 4	(a)	75° F. Room temp.	65	3.0	84	49	16
	(b)	90° F. Hot room	57	2.6	72	33	28

In the course of these experiments it was early noticed that only a small proportion of the rats, which had been inoculated with a virulent plague culture and then placed along with the fleas in the cold room, developed septicaemia. We, therefore, in a series of four experiments further investigated the influence of temperature on the disease in the rat with special reference to the development of septicaemia. The technique was as follows. In each series a number of rats caught in

Bombay city were inoculated, each with the same amount of the same culture of plague bacilli. Some were kept at room temperature, while others were kept, in the case of three of the experiments in the cool room at varying temperatures, and in the case of the fourth experiment in the hot room about 90° F. As soon after death as possible the rats were examined. In the case of those which had died from plague a careful microscopical examination of the blood was made and the presence or absence of plague bacilli was noted. If present it was recorded whether they were abundant or only few in number.

The data of these series of experiments are given in Table VI.

A study of this table shows us that low temperatures affect plague infected rats in such a manner that they die more quickly, and do not develop septicaemia to such an extent as rats kept at higher temperatures. It is also seen that the lower the temperature at which the rats are kept the more marked is this action. Further, from series 4 of these experiments it would appear that a high temperature such as 90° F. has little or no influence on the disease in the rat¹.

We have, therefore, obtained experimental evidence which goes to show that a low mean temperature, such as 50° F., would have a limiting influence on a plague epidemic, inasmuch as fewer rats would develop a good septicaemia and in consequence fewer fleas would have an opportunity of taking plague bacilli into their stomachs.

Conclusions as to the direct influence of temperature on the seasonal prevalence of plague.

1. *A plague epidemic is checked when the mean daily temperature passes above 80° F. and especially when it reaches to 85° F. or 90° F.*

2. *A mean temperature above 80° F. affects the conditions to which the plague bacillus is subjected in the stomach of the flea. At high temperatures about 90° F. the plague bacilli disappear from the stomach of the flea much more quickly than at lower temperatures, namely, between 70° and 80° F. Fleas remain infective for a much longer time at the lower temperature.*

3. *A plague epidemic may, however, come to an end when the temperature is most suitable. Other factors must, therefore, be present in these cases.*

¹ It must be remembered in this connection that the absence of a well-marked septicaemia *post mortem* may be the result of the low temperature inhibiting multiplication of bacilli in the blood of the rat after death.

4. A mean temperature about 50° F. may have a direct influence on the seasonal prevalence of plague. At such a temperature the number of plague infected rats which develop septicaemia is very much less than at high temperatures. The lower the temperature the fewer rats become septicaemic. A very high mean temperature seems to have little or no influence in the reverse direction.

V. SEASONAL VARIATIONS IN THE LIFE HISTORY AND HABITS OF THE RAT.

(a) *Breeding of rats.*

It is possible that climatic conditions might affect the breeding season of these rodents and in this way indirectly influence outbreaks of plague. We have made observations on this point both in the Punjab and in Bombay.

(1) *Punjab.*

The detailed figures have been already published (vol. vii. p. 906 and chart). The main result is that the rats breed all the year round but with more vigour at some seasons than at others. In the winter months, November to February, the months just preceding the plague epizootic season, breeding is at its lowest. While during the rest of the year the percentage of pregnant females to total females is always about or above the mean, there seem to be three months, namely April, September and October, in which breeding is most vigorous. In this connection it may be remarked that the mean temperature of April does not differ much from that of September and October, namely about 80° F. The plague season in this part of India falls between February—June. It is evident, therefore, that any loss in the rat population caused by an epizootic during this season would be amply made up by the addition of a large number of young susceptible animals before the next outbreak took place.

(2) *Bombay.*

In Bombay we have investigated this question of the breeding of rats both in the case of *M. decumanus* and in the case of *M. rattus*. The results have been given already (vol. vii. pp. 748–9 and charts). The main facts are that *M. decumanus* breeds all the year round. In

the months of December, January and February, that is to say at the beginning of the epizootic, the percentage of pregnant females to adult females is lower than at any other time. During the rest of the year there seem to be three seasons at which breeding is most vigorous, namely, March, July—August and October. The curve showing the percentage of young to adult rats follows the 'pregnant curve' very closely. The plague epizootic amongst *M. decumanus* begins in January, is raging during February and March and rapidly declines in April. While, therefore, there is no direct relationship between the breeding season of this species and the plague epizootic, there is no question that between the end of one epizootic and the beginning of the next one there would be a large number of young susceptible individuals added to the rat population.

M. rattus in Bombay, as in the Punjab, breeds all the year. During the months December to March the percentage of pregnant females to adult females is lowest, and reaches its maximum during the months July to September. The curve showing the percentage of young rats to adult rats follows the 'pregnant curve' very closely. When we come to the epizootic curve we find that plague begins in January, increases during February, is at its maximum in March and April and declines during May. The chief breeding season in Bombay for *M. rattus* is, therefore, during the non-epizootic season. It is also seen that before the epizootic there is a large addition of young animals to the population.

(b) *Habits of rats.* It is further possible that the habits of the rat might be so affected by varying climatic conditions, as to influence the seasonal prevalence of the disease. We have made observations on this point, both in Bombay and in the Punjab over a period of 18 months, with negative results.

In Bombay city we have closely observed the habits of these rodents and at no time of the year have we noticed any change in them. Further, both in the four villages in the neighbourhood of Bombay and in the villages of Kasel and Dhand in the Punjab, in all of which villages rats were systematically trapped by us for over a year, we have never observed any migration from the houses to the fields nor any other seasonal change of habit. In the case of the Punjab villages it has been suggested that the decline of a plague epidemic may be due to the migration of the rats to the fields at a time when the crops are being cut. Now in Kasel at the height of the epidemic, when rats were dying in large numbers in the village, the crops had just recently been cut. There had evidently been no migration of the rats to the fields,

and no migration was noticed. We can, therefore, only conclude that, as far as we have investigated the subject, climatic conditions appear to have no influence on the habits of rats in any way that might influence the seasonal prevalence of plague.

(c) *Seasonal variations in the total rat population.* Apart from breeding, plague epizootics would appear to be the chief factor which might produce considerable variations in the total rat population. In a city like Bombay where the rat population is enormous it would be impossible to appraise with any accuracy the effect of the plague epizootic on its numbers. The general result however must be that there is some diminution in the total number of rats.

However, in the two villages of Dhand and Kasel in the Punjab some observations in the above directions were made, which observations, although by no means conclusive, seem to support this conclusion.

The result of the systematic trapping month by month in Dhand is shown in Table VII. During the two months before the plague epizootic began over 1500 rats were caught and killed. Plague amongst the rats appeared in February and lasted till about the middle of April. As far as could be judged from the number of dead rats found, 46 in all, the outbreak was not very severe, possibly due to the

TABLE VII. *Dhand.*

Month	No. of traps set	Total no. of rats taken	Average no. of rats per 100 traps	Remarks
December	943	742	80	
January	2393	801	34	
February	1699	258	15	Epizootic began.
March	1492	141	10	Epizootic continued.
April	436	21	5	Epizootic stopped.
May	534	17	3	
June	936	28	3	
July	—	—	—	No traps set.
August	—	—	—	No traps set.
September	233	78	34	
October	301	149	50	
November	875	283	32	

diminution in the rat population brought about by the previous trapping. During the epizootic trapping still went on but very few rats were taken. After the outbreak had come to an end, catching continued but with very poor results, only 45 animals being taken during May and June. No traps were set during July and August. Operations were begun again in September, when, as will be seen from

the table, the number of rats had evidently increased considerably, as the takes during the next three months were about equal to what they were when the operations were first begun. There is no doubt that even before the epizootic began the rats in Dhand had been considerably diminished in number on account of the trapping. We cannot, therefore, attribute the great diminution at the end of the plague outbreak, which was readily acknowledged by the people themselves, to the epizootic alone. The important part, however, of the observations is that between the time when the epizootic ended in April and October the rat population had evidently again increased to a number as great, if not greater, than what it was when the epizootic began.

TABLE VIII. *Kasel.*

Month	No. of traps set	Total no. of rats taken	Average no. of rats per 100 traps	Remarks
December	782	1086	138	
January	Nil	Nil	Nil	Trapping stopped.
February	448	398	89	Trapping began again on
March	1260	870	69	20. ii. 06.
April	586	316	54	Epizootic began.
May	927	315	34	Epizootic continued.
June	1048	202	19	Epizootic stopped.
July	772	132	17	
August	774	232	30	
September	619	292	47	
October	531	215	40	
November	854	587	69	

In Kasel very similar observations were made. It will be seen from Table VIII, that during the first month of trapping a large number of rats were taken, over 1000 being removed from the village. After the village had been once trapped systematically, house by house, the operations were suspended for about six weeks, being begun again on the 20th February. In March, the month before plague began, an average of 69 rats per 100 traps was obtained. During the epizootic which lasted from April to June, and which was evidently fairly severe, as over 300 dead plague infected rats were found, this average gradually diminished, until in July, the month after the epizootic had ceased, only 17 rats per 100 traps were caught. That the removal of some rats by trapping was not altogether responsible for this diminution is shown by the observation that, although breeding was no more vigorous than during the epizootic, the number of takes began to rise in August, and went on increasing, until by December as many rats were caught as at the time when plague started.

It appears to us, therefore, that we have here evidence supporting the hypothesis that a plague epizootic may greatly diminish the number of rats for the time being, but that after the epizootic has stopped, the population may soon assume its usual proportions.

(d) *Seasonal variations in the proportion of immune to susceptible individuals amongst the rat population.*

It is *a priori* justifiable to infer that during a plague epizootic a number of rats suffer from a mild attack of the disease and, recovering therefrom, are more or less immune to a second attack.

This hypothesis receives a certain amount of support from a comparison of the flea transmission experiments done with Bombay rats at room temperature in the plague season with similar experiments done at the beginning of the non-plague season in the cool room at 70° F. That the conditions of experiment in the latter series were otherwise suitable for successful results is borne out by the fact that a series of guinea-pig experiments carried out under exactly the same conditions yielded 100 p.c. of successes.

Table IX contains the details of these observations, the experiments with guinea-pigs being put in for the sake of comparison. From this

TABLE IX.

Animals	Season of year	Temperature at which kept	No. of experiments completed	No. of successful transmissions	P.c. of successful transmissions
Bombay rats	Jan.—March 1906	Room temp. 73—78° F.	29	16	55
	July—August 1906	Cool room 70° F.	46	13	28
Guinea-pigs	March—April 1906	Room temp. 75—78° F.	14	13	93
	July—August 1906	Cool room 70° F.	15	15	100

table it is seen that in the plague season 55 p.c. of the rats exposed to infection contracted the disease, while after the epizootic had come to an end only 28 p.c. became infected. It is to be remembered that the rats were in each instance got in the city of Bombay, no distinction being made between *M. decumanus* and *M. rattus*. Further, we have stated that the conditions were as far as possible the same in both series of experiments and were certainly, as shown by the guinea-pig results, not less favourable in the series made at the end of the epizootic.

The hypothesis gains further support from the results of an analysis of the post-mortem findings in rats which died of plague during the epizootic and in rats which died in the non-plague season. In Table X are set forth the results of the microscopic examination of the blood, as

TABLE X.

Season of year	P.c. of rats whose blood contained no plague bacilli	P.c. of rats whose blood contained only a few plague bacilli	P.c. of rats whose blood contained abundant plague bacilli
Plague epizootic season	13·5	53·4	33·1
Non-epizootic season	56·7	32·0	11·3

regards the degree of septicaemia present, in two series of rats which had been found dead of plague in Bombay city, namely,

(a) a series of 1000 rats found during the plague epizootic, and
 (b) a series of 900 rats found during the non-epizootic season, namely, from 30th May to 30th October 1906. From this table it is seen that in the non-epizootic season a much larger number of rats died from plague without any bacilli appearing in the blood than during the epizootic, and *vice versa* the number which developed a good septicaemia was much greater in the epizootic season than in the non-epizootic season. We take it that this pathological difference points to a greater number of rats possessing a certain amount of resistance to the disease after the epizootic than during the epizootic season.

As far as they go, therefore, these observations seem to show that there is a greater proportion of immune to susceptible rats in Bombay at the end than at the beginning of the plague epizootic.

VI. INFLUENCE OF CLIMATIC CONDITIONS ON THE HABITS OF MAN.

It is now generally accepted that the infection of plague is in the buildings, and that huts, rooms or houses in which plague has occurred amongst rats are during an epidemic highly infectious. Further, the infection clings to these localities even after they have been evacuated, so that individuals returning to them have promptly contracted the disease. This infectivity of houses is at once explained on the basis of the rat-flea-man theory. The infection will be kept up as long as the plague mortality amongst the rats continues. Rats dying in the open outside the houses would be less dangerous inasmuch as the fleas on leaving them would soon be destroyed or dispersed by sunlight. Accepting, therefore, that the infection of plague is wholly within the

houses, we can imagine that, if owing to climatic conditions the inhabitants were accustomed to evacuate their buildings and live in the open at any particular season, this habit would influence the seasonal prevalence of the disease. We can conceive that a plague epidemic might be brought to an end by such a migration. There is no doubt that in many of the villages of the Punjab, as happened at Kasel while it was under our observation, the plague epidemic, occurring as it does at the beginning of the hot weather when the people are cutting the crops, may be limited by partial evacuation. This evacuation, however, is not at all general and cannot be said to have any influence otherwise than in limiting somewhat the number of attacks.

Further, in Bombay no general seasonal evacuation of houses can be said to occur. There is no doubt that more people sleep out of doors in the hot weather than in the cold, but the difference is so insignificant as to be negligible.

In Poona we have seen that the plague epidemic season is as a rule in the autumn, coming to an end about December. At this time, when the mean temperature is comparatively low, there can be no suggestion of the people living in the open. The same holds good for Rawalpindi, in which place, as we have seen, the epidemics decline at the beginning of the cold weather, at a time when the inhabitants are living indoors.

A survey of the whole subject, therefore, leads us to the conclusion that those habits of the people, which are dependent upon varying climatic conditions, have no influence on the seasonal prevalence of plague.

VII. VARIATION IN THE VIRULENCE OF THE BACILLUS.

We have shown (vol. vi. pp. 496, 502) that, contrary to the results got by some other observers, the plague bacillus does not become diminished in virulence by passage through rats. In one series of experiments, in which the subcutaneous method of injection was used, twenty-six passages from rat to rat were effected without recourse to cultivation on artificial media. No alteration in the virulence of the bacillus was brought about by these passages. In another series of experiments, in which the cutaneous or rubbing-in method of inoculation was employed, again no alteration of virulence was observed after twenty-six passages.

We have also shown (vol. vii. p. 352), that the plague bacillus isolated from rats during the off plague season in Bombay is of full

virulence. There appears to be no difference in the virulence of the plague organism when isolated from naturally infected rats at any season of the year in Bombay.

These observations, therefore, appear to us to warrant the conclusion that variation in the virulence of the plague bacillus plays no part in determining the seasonal prevalence of the disease.

VIII. SEASONAL VARIATIONS IN THE LIFE HISTORY AND HABITS OF RAT FLEAS.

(a) *Influence of temperature on breeding.* We have already detailed experiments (see above, p. 243) which show that a high mean temperature affects the breeding of fleas to a considerable extent, in that it appears not only to restrain the imago from depositing eggs, but also to be deleterious to the development of the eggs into larvae. It is well known that quite cool weather materially extends the time required for the complete metamorphosis.

(b) *Seasonal variation in the number of rat fleas.*

While it is *a priori* certain that the number of rat fleas present must have an influence on a plague epizootic, it has been shown experimentally in the course of the godown experiments (vol. vii. p. 428) that, other conditions being the same the rate of progress of a plague epizootic amongst the guinea-pigs was much slower in those godowns in which the flea infestation was slight than in the godowns which were abundantly supplied with fleas. The questions, therefore, to be now answered are (a) is there in nature a seasonal variation in the number of rat fleas? and (b) if so, does it correspond to the rise and fall of the plague epidemic? Answers to these questions were sought for both in Bombay and in the Punjab villages.

1. *Observations in Bombay.*

In Bombay it is notorious that fleas are more common at one season of the year than at another. The season of greatest prevalence is generally stated to be during March, April and May. The observations on which these statements are based probably refer to *Pulex irritans* or to *Pulex felis* and they are not supported by any definite figures.

In order to obtain more accurate data regarding *P. cheopis*, two methods were adopted by us:

(A) Thirty houses in different parts of Bombay city were selected. These houses had at one time when plague infected come under our observation and were therefore known to be rat infested. When the present observations were made they were free from plague. At intervals of about two months two guinea-pigs were placed in each house and left in over night. They were then chloroformed and searched for fleas, which were carefully enumerated. Between June 1906 and May 1907 seven countings were made in each house, and the data obtained are given in Table XI and chart. These seem to indicate that there is a distinct

TABLE XI.

Season when observations were made	P.c. of total houses which contained any fleas	Average number of fleas per house
End of June 1906	24	0·4
Beginning of October 1906	26·6	1·3
End of November 1906	43·3	1·7
Middle of January 1907	60	3·8
Beginning of March 1907	72·4	4·9
Middle of April 1907	59	2·5
Beginning of May 1907	46	2·3

seasonal variation in the number of rat fleas, the largest number being found from January to April or May, with a maximum prevalence in March.

(B) In the description of the methods which were used for studying the epizootic we indicated (vol. vii. p. 738) the means which were adopted to obtain as accurate a census as possible of the fleas infesting the rats. Traps were set daily in different situations in the various districts of the city. Immediately after it was found the trap was enclosed in a stout canvas bag. When the traps arrived at the laboratory, each one was removed from the bag and both traps and bag were at once put into a tin box to which chloroform was added. Four of these boxes were in constant use. Each of them had a metal tray resting on the bottom. After the rats were killed by the chloroform the tray and its contents were removed 'en bloc.' The fleas on the tray were then counted, after those in the bag had been shaken out. At the same time each rat was separately searched and the fleas found on it added to the total.

From 1st November 1906 to 30th October 1907, on an average between 100 and 200 rats were daily dealt with in this manner so that the data, which we are now about to present, are founded on figures sufficiently large to minimise the error inseparable from the method.

A summary of the data month by month is given in Table XII and Chart VII. In the case of *M. rattus*, the number of fleas is above the mean during the months, February—May, reaching a maximum in March and April. The same may be said of the fleas infesting *M. decumanus* with the slight difference that the mean line is crossed in January. During the rest of the year, namely June to January, the number of fleas on both species is below the mean, being lowest during the months September—December. These results coincide remarkably with

TABLE XII. *Flea prevalence in Bombay.*

Month	<i>Mus rattus</i> ¹			<i>Mus decumanus</i> ²			All rats ³		
	Total no. of rats	Total no. of fleas	Average no. of fleas per rat	Total no. of rats	Total no. of fleas	Average no. of fleas per rat	Total no. of rats	Total no. of fleas	Average no. of fleas per rat
1906 November	1,313	3,231	2·5	410	2,348	5·7	3,483	11,264	3·2
December	2,087	5,362	2·6	489	2,803	5·7	4,591	14,429	3·1
1907 January	1,927	6,150	3·2	465	4,227	9·0	4,371	19,648	4·5
February	1,693	7,689	4·5	309	3,666	11·9	3,283	18,362	5·6
March	1,799	9,345	5·2	300	3,838	12·8	4,425	29,814	6·7
April	1,911	9,981	5·2	306	4,250	13·9	4,032	27,478	6·8
May	1,721	7,972	4·6	421	5,043	12·0	3,752	21,589	5·8
June	1,426	4,893	3·4	331	2,718	8·2	3,713	16,888	4·5
July	1,118	4,291	3·8	305	2,139	7·0	3,746	16,083	4·3
August	767	2,766	3·6	198	1,358	6·9	3,216	12,604	3·9
September	1,116	3,722	3·3	326	1,361	4·2	4,018	12,782	3·2
October	1,555	4,232	2·7	327	1,607	4·9	5,183	15,041	2·9

the conclusions which have been tentatively drawn from the figures obtained by the other method. There can, therefore, be little doubt that there is a seasonal variation in the number of rat fleas in Bombay and that the months February to May are those in which these insects are most numerous, the maximum being reached in March and April. We have now to co-relate this seasonal variation of rat fleas with the seasonal prevalence of the plague epidemic. The epidemic season of plague in Bombay, as regards both the rat and man, is from January or February to April or May, the maximum prevalence being either in March or April. It is apparent, therefore, as far as Bombay is concerned,

¹ The traps contained only *Mus rattus*.

² The traps contained only *Mus decumanus*.

³ All traps containing *M. rattus*, *M. decumanus*, either alone or together with a few *Nesokia* and mice.

that the plague season is contemporaneous with the season of prevalence of rat fleas, and that during the off plague season the number of rat fleas is well below the mean.

(2) *Observations in the Punjab.*

During the year that the observations were in progress in the Punjab villages of Dhand and Kasel a census of the fleas infesting the rats was made. The method adopted was the same as has been already described for Bombay, only rats caught alive being used.

Two species of fleas were found on the rats, namely, *Ceratophyllus fasciatus* and *P. cheopis*.

(a) *Ceratophyllus fasciatus.*

Only about 2% of all the fleas taken belonged to this species. This flea had a very definite seasonal prevalence in the villages investigated (see vol. vii. p. 917). When the observations were begun in December 1905 *Ceratophyllus fasciatus* was found to be present on the rats in both villages. It disappeared from Dhand about the end of March and from Kasel about the middle of April, except for an isolated specimen found in May. From this date no fleas of this species were found till the first week in November when they reappeared almost simultaneously in both villages. They remained present until trapping was stopped in the first week in December.

(b) *P. cheopis.*

P. cheopis is the common rat flea in the Punjab as it is in Bombay. The data obtained regarding the seasonal variation in its numbers have been already given (vol. vii. p. 916 and chart). They show that the number of *P. cheopis* is above the mean from November to May with a maximum probably in April. During the remaining months—June to September—the flea prevalence is below the mean, the absolute minimum being reached in August and September when the number per rat is six times less than in April.

Now the plague season in the Amritsar district of the Punjab in which the villages are situated is from February to May inclusive. It will also be remembered that in the year 1906, plague was present in Dhand from 27th January to 21st April and in Kasel from 2nd April to 17th July, reaching its maximum during the first half of May. By the beginning of June the epidemic was practically over, after that date

only a few scattered cases occurring. The seasonal prevalence of *P. cheopis* therefore corresponds fairly closely with the plague mortality. The connection may, perhaps, best be stated in saying that the non-plague season corresponds to the months when the average number of fleas per rat is below the mean, while the epidemic season corresponds to the period when the average number of fleas per rat is above the mean.

TABLE XIII. *Flea prevalence in Dhand and Kasel (Punjab).*

Month	Total no. of rats on which fleas were recorded	Total no. of fleas	Average no. of fleas per rat
December 1905	1631	12024	7.4
January 1906	809	9085	11.2
February „	647	6258	9.7
March „	1020	8069	7.9
April „	350	4396	12.6
May „	341	2482	7.3
June „	232	1131	4.9
July „	136	514	3.8
August „	226	447	2
September „	374	853	2.3
October „	375	1955	5.2
November „	867	6780	7.8

The epizootic continued in Dhand from February to April, and in Kasel from April to June.

SUMMARY.

1. *Both in Bombay city and in the Amritsar district of the Punjab there is a distinct seasonal variation in the number of rat fleas.*

2. *In these places this seasonal variation of rat fleas corresponds directly in a general way with the plague mortality. During the season when plague is epidemic the average number of fleas per rat is above the mean, while during the non-epidemic season it is below the mean. The height of the epidemic corresponds fairly closely with the season of maximum flea prevalence.*

IX. GENERAL SUMMARY AND CONCLUSIONS.

I. The seasonal prevalence of plague has been studied in six different places in India. These places are widely separated from one another and are under the influence of quite different climatic conditions. It was found that plague, once it has become established tends to

recur every year at the same season. The plague season was found to vary in different places.

II. The possible factors which might influence the seasonal prevalence of plague were considered. Three factors have been indicated which influence the rise and fall of the epidemic as follows:

A. *Temperature.*

1. *Epidemiological facts.* From a study of the mean temperature in co-relation with the plague mortality in six widely separated places in India, it appears that plague cannot exist in epidemic form in any of these places when the daily mean temperature is as high as 85° F. and over. If an epidemic is in progress, as soon as or very shortly after the temperature has reached the above height or even less, the plague epidemic receives a check and rapidly declines. While this is so, it would also appear that epidemics may come to an end when the temperature is most suitable. Another factor or factors must, therefore, be in operation in these instances.

2. *Experimental data.* At all seasons of the year experiments were carried out in the godowns and in cages in the laboratory. Specially constructed hot and cold chambers were also used. These experiments showed (a) a mean temperature, of 85° F. and over, affects the fate of the plague bacillus in the stomach of the flea. At this temperature fewer successful transmissions from animal to animal are obtained and besides the flea does not retain its power of infecting nearly so long as it does at a lower temperature, *i.e.*, 70° F. At the higher temperature the plague bacilli disappear from the stomach of the flea much more quickly than at the lower temperature.

(b) While a mean temperature high in comparison with 70° F. has no effect on the number of plague infected rats which contain bacilli in the blood at death, at a low mean temperature, such as 50° F., the number of infected rats which die before bacilli appear in the blood is much greater than at the higher temperature, *i.e.* 70° F.

B. *Variation in the total number and in the susceptibility of rats.*

Evidence has been adduced to show that a plague epizootic considerably diminishes for the time being the total number of rats present in a place. There is also evidence to show that the effect of a plague epizootic is to increase the proportion of immune to susceptible rats. It is clear that these two factors would have an influence in limiting and in bringing to an end plague amongst the rats.

It was found both in Bombay and in the two Punjab villages that breeding of rats goes on all the year round, but that it is especially vigorous during the season between the end of one epizootic and the beginning of the next. During this interval there would therefore be added to the rat population a large number of young susceptible individuals, a factor which would evidently influence the rise of the epidemic.

C. Seasonal variation in the number of rat fleas.

It has been shown both in Bombay and in the Punjab that there is a seasonal variation in the number of rat fleas. And further it has been found that the plague epidemic season corresponds with the season of greatest flea prevalence, while during the months when plague is at its minimum fleas are fewest in number.

III. In conclusion we may state the following propositions:—

The rise of the rat epizootic and in consequence the human epidemic depends upon:

(a) A suitable mean temperature, somewhat below 85° F. and in general over 50° F.

(b) A sufficient number of susceptible rats.

(c) A sufficient number of rat fleas.

The fall of the rat epizootic and in consequence of the epidemic, is determined by some or all of the following factors:

(a) A high mean temperature, 85° F. and above.

(b) A diminution in the total number of rats and an increase in the proportion of immune to susceptible animals.

(c) A diminution in the number of rat fleas.

XXXII. ON THE DIFFERENTIAL DIAGNOSIS OF THE PLAGUE BACILLUS FROM CERTAIN ALLIED OR- GANISMS.

It was felt desirable that an investigation should be made of the characters of *B. pestis*, and especially of the best means to be adopted in distinguishing it from certain allied organisms liable to be mistaken for it. The tests we have selected as aids in arriving at a diagnosis will be found tabulated below, but a few supplementary remarks may be added on several points which do not lend themselves to a summary description.

It will readily appear from a consideration of the Table that the organisms we have examined may be classified into 4 groups:—1st—the plague bacillus both virulent and avirulent; 2nd—certain strains of the *B. pseudotuberculosis rodentium*; 3rd—5 organisms belonging to the “haemorrhagic septicaemia” group; and 4th—organisms conforming to the type of *B. enteritidis* (Gaertner).

I. BACILLUS PESTIS.

Appearances of growth on neutral agar. For diagnostic purposes cultures on dry neutral agar are fairly characteristic. Early cultures consist of delicate dew-drop-like colonies, so that the growth when looked at by reflected light somewhat resembles a ground-glass surface,—indeed, this appearance is retained even in old cultures. After several weeks' growth, isolated colonies by transmitted light are seen to be composed of a central, brownish, opaque nucleus surrounded by a thinner, translucent, granular zone; the nucleus may be eccentric. The central part has a well-defined outline, but that of the entire colony is crenulated. If a culture one month old be examined with a hand-lens, on each colony there may be seen scattered over the peripheral zone fine dew-drop-like projections giving the colony a distinctly granular look to the naked eye. Secondary colonies are frequently seen in

cultures two or three weeks old; these have a well-defined margin and contain a central thicker nucleus.

The stalactite test. The first essential towards obtaining good stalactites in a plague culture is absolute lack of vibration of the shelf on which the flask stands. To ensure this it is a good plan to have the shelves fixed to beams in the wall, instead of being supported on the floor. It is convenient also that each shelf should be separated from the wall by a space about a foot wide so that a candle can be placed behind the flask while it is being examined. The contrivance of floating pieces of cork in the broth cannot be recommended, simply because it is quite unnecessary. The addition of oil to assist stalactite formation is an advantage, but even without oil good stalactites can be obtained. When a culture is made fresh from the body stalactites may not readily form; the flask should then be shaken and its contents allowed to settle.

A highly characteristic appearance is obtained when a small quantity, *e.g.* 1 c.c. of blood containing say 10 to 100 bacilli per c.c., is inoculated into a 100 c.c. flask of neutral broth. The plasma forms a soft clot dispersed throughout the broth and if the flask be kept undisturbed each bacillus ultimately gives rise to a tack-like growth enclosed in a similarly shaped cavity. The head of the tack corresponds to the original point of growth, the vertical portion being formed by a down growth of the bacilli from this point; the cavity is presumably due to a solution of the fibrin by the bacilli.

In a typical stalactite growth the broth is clear. A difference in this respect was noted in the avirulent culture in which the broth was somewhat turbid. Again, the stalactites of the avirulent strain were not so characteristic as those of the virulent culture, *i.e.* they were not so uniformly thin and long.

Fermentation tests. MacConkey (1905) has recently pointed out that *B. pestis* and *B. pseudotuberculosis rodentium* produce parallel reactions in media containing fermentable substances, *viz.* the production of acid but no gas in glucose, laevulose, galactose, maltose, mannite and dextrin—lactose, cane sugar, and dulcite remaining unchanged. As will be seen from the table we have confirmed some of these reactions and find that they hold good in the case of an avirulent strain of plague isolated probably 7 years ago.

Animal tests. We do not propose to enter here into the special diagnostic points in the pathology of plague, but naturally the presence or absence of such a characteristic phenomenon as the involution forms in animal tissues is of great value in deciding as to whether or not a doubtful organism is the plague bacillus.

TABLE.

Name	Origin and where obtained	Neutral agar	Glucose	Lactose	Dulcitate	Laevalose	Mannite	Galactose	Stalactite test	Animal test
<i>B. pestis</i> (virulent)	From guinea-pig Bombay		Acid; no gas	-	-	Acid; no gas	Acid; no gas	Acid; no gas	Typical	Pathogenic to rats and guinea-pigs.
" (avirulent)	Probably from Bombay—7 years old (Dean)		Acid; no gas	-	-	Acid; no gas	Acid; no gas	Acid; no gas	Typical, less so than virulent	Non-pathogenic to rats and guinea-pigs.
<i>Schweineseuche bacillus</i> I.	(Wassermann) Pasteur Institute	Like <i>B. pestis</i>	No growth	No growth	No growth	No growth	No growth	No growth	Very fair stalactites	
" II.	do.	"	"	"	"	"	"	"	Good stalactites	
" III.	do.	"	"	"	"	"	"	"	Suggestion of stalactites	
<i>Pasteurellose ovine</i>	(Lignières) Pasteur Institute	"	"	"	"	"	"	"	Negative	
" equine	do.	"	"	"	"	"	"	"	"	
<i>B. suisepitiscus</i>	(Preis: Origin A) Pasteur Institute	"	"	"	"	"	"	"	Very short thick stalactites	
"	(Preis: Origin P) Pasteur Institute	"	"	"	"	"	"	"	Tendency to stalactites	
<i>B. of fowl cholera</i>	Pasteur Institute	"	"	"	"	"	"	"	Good stalactites	
"	Bombay	"	"	"	"	"	"	"	Negative	
<i>B. pseudotuberculosis</i> (Pfeiffer A.)	More than 10 years old Pasteur Institute	Growth too luxuriant	Acid; no gas	-	-	Acid; no gas	Acid; no gas	Acid; no gas	Very fair stalactites	Killed g.-pigs acutely by cutaneous method.
<i>B. pseudotuberculosis</i> (Pfeiffer K.)	Pasteur Institute	"	"	-	-	"	"	"	Short thick stalactites	
<i>B. pseudotuberculosis</i> (Pfeiffer)	Lister Institute	Like <i>B. pestis</i>	"	-	-	"	"	"	Very good stalactites	
<i>B. pseudotuberculosis</i> (Singe Bi)	(Binot) Pasteur Institute	Growth too luxuriant	"	-	-	"	"	"	Short thick stalactites	
<i>B. tuberculose zoogléique</i>	(Nicolle) Pasteur Institute	"	"	-	-	"	"	"	Negative	
<i>B. pseudotuberculose coccobacillaire</i>	(Borrel) Pasteur Institute	"	"	-	-	"	"	"	"	
Organism causing plague-like appearances in guinea-pigs	Bombay	"	Acid + gas	Acid + gas	Acid + gas	Acid + gas	Acid + gas	Acid + gas	"	Produced an infection in guinea-pigs by cutaneous method.
Same as above	Bombay	"	Acid + gas	Acid + gas	Acid + gas	Acid + gas	Acid + gas	Acid + gas	"	
<i>B. suispestifer</i> (Kruse)	Pasteur Institute	"	Acid; no gas	-	-	Acid; no gas	Acid; no gas	Acid; no gas	"	

Note:—The media in the above table containing sugars &c. were prepared by the methods recommended by MacConkey.

II. *BACILLUS PSEUDOTUBERCULOSIS RODENTII*.

The growth on agar of most of the races we have examined was more vigorous than that of *B. pestis* though a culture obtained from the Lister Institute resembled plague very closely.

The tests for stalactites gave interesting results. The culture just mentioned gave undoubted long stalactites, reaching almost to the bottom of the flask, indistinguishable indeed from those of the plague bacillus—the broth being fairly clear though later it became decidedly cloudy. *B. pseudotuberculosis* (Pfeiffer) A. grew very fair stalactites which however took a long time to develop (3 weeks). *B. pseudotuberculosis* (Binot) showed in about a fortnight very thick short atypical stalactites; while *B. pseudotuberculosis* (Pfeiffer) K. developed short thick stalactites in about 48 hours.

The various sugar reactions coincided with those of *B. pestis* as mentioned already. Fortunately we have never encountered this organism in our stock of experimental guinea-pigs. In such an event, we should rely chiefly on the effects of the injection of the culture into several *white* rats.

III. BACILLI OF THE "HAEMORRHAGIC SEPTICAEMIA" GROUP.

The cultures of all the organisms in this group tested by us gave a distinctly more delicate growth on neutral agar than the plague bacillus though otherwise resembling it.

Stalactite test. The bacillus of German swine plague (I) formed good stalactites in 3 days though later the broth showed clumps. No. II also gave good stalactites indistinguishable from those of plague but in this case their formation was delayed for at least 3 weeks. *B. suisepiticus* of Preisz A. and P. showed very short thick stalactites after a fortnight's growth. Stalactites formed in a culture of the fowl-cholera bacillus obtained from the Pasteur Institute, which could not be distinguished from those of *B. pestis*; on the other hand a recently isolated strain failed to produce them.

It is noteworthy that of all the cultures tested for the production of stalactites only those included in this group and certain strains of the pseudotubercle bacillus showed any tendency to their formation—a fact

which serves to confirm the close relationship of these organisms to the bacillus of plague.

Sugar media test. The differentiating test of this group was furnished by the employment of MacConkey's sodium taurocholate medium. We failed to obtain any growth of these organisms in various modifications of this medium.

Animal tests. An opportunity was afforded of testing by the cutaneous method the effect on guinea-pigs of a spleen from a goose recently dead of a haemorrhagic septicaemia. The spleen was rubbed into a shaved area of 2 guinea-pigs weighing about 200 grammes each. One died in 26 hours showing *post-mortem* a local cutaneous reaction, marked general subcutaneous oedema, a considerable amount of fairly clear peritoneal and pleural effusion, and congestion of the inguinal glands. The spleen was not enlarged. On microscopical examination no bacilli were seen in the spleen, but small organisms showing bipolar staining were noted in the heart blood. A good culture was obtained from the heart blood on agar and in broth. The second guinea-pig died in 3 days showing an intense local reaction, general subcutaneous injection, small double inguinal, pelvic and axillary buboes, and pleural and peritoneal effusion. The spleen was not much enlarged and showed no nodules but contained fairly numerous organisms. The heart blood showed numerous very small diplo-bacilli, and gave a good culture on agar. The glands showed a few bacilli. These results correspond with those obtained by Fritsche.

IV. BACILLI OF THE *B. ENTERITIDIS* (GAERTNER) GROUP.

1. *B. suispestifer* (Kruse) is unlikely to be mistaken for *B. pestis*. Its growth on agar is too vigorous and opalescent, and the colonies never assume the typical appearances of those of the plague bacillus. The test for stalactites proved negative. The sugar reactions carried out by us differed from those of *B. enteritidis* (Gaert.) in that no gas development was observed in glucose, mannite and laevulose.

2. Another organism belonging to this group is of practical importance in our work as it was the cause of an epizootic amongst the young stock guinea-pigs. The pathological features of the disease markedly resembled those of plague. Smears of the spleen after death frequently showed bipolar-stained bacilli very like *B. pestis*. It may be mentioned that the disease was given to a healthy guinea-pig by cutaneous inocu-

lation, the spleen of an animal showing numerous nodules being used for the purpose.

A similar organism has been isolated on one or two occasions from rats brought dead to the Laboratory for examination. On two occasions the spleen of a rat which did not prove to be plague infected when inoculated cutaneously into a guinea-pig gave rise to a subacute infection. In one case an inguinal gland was enlarged and the spleen contained a nodule the size of a millet seed.

From the foregoing details it may be asserted that organisms of the "Gaertner" type occasionally occur in rats, and that if the cutaneous method in guinea-pigs be employed for purposes of diagnosis, an infection by this organism may result; confusion from this cause may easily be avoided if the possibility of such an infection be remembered.

Into this group, or more broadly into the *coli* group, probably fall many of the so-called plague-like bacilli described by various authors. We can merely state our experience that we have never encountered any organism of the group which with ordinary care is in the least likely to be mistaken for *B. pestis*.

SUMMARY.

We have no desire to claim for the tests described above that they will be found applicable to every strain of organism belonging to any particular group. Our experiments were necessarily limited to a few races of each—many of these being probably avirulent from long subcultivation on artificial media. We merely wish to indicate the general methods we would adopt in any case of difficult bacteriological diagnosis, although as a matter of fact the only tests we found it necessary to use in the course of our work were the method of cutaneous and subcutaneous inoculation into animals and the stalactite test.

The tests referred to in this paper may be summarised thus:

1. The plague bacillus gives a fairly characteristic type of colony on neutral agar; it forms typical stalactites in neutral broth; it gives certain definite fermentation reactions.

2. *B. pseudotuberculosis* resembles *B. pestis* more closely than any other organism. The animal test on white rats is probably the best for its differentiation.

3. Bacilli of the haemorrhagic septicaemia group appear to be inhibited in their growth in media containing sodium taurocholate.

4. Bacilli of the *B. enteritidis* (Gaertner) group are the most readily distinguished of all; the appearance of agar cultures, the negative stalactite test, and their fairly definite fermentation sugar reactions are sufficient to mark them off from the plague bacillus.

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MACCONKEY, A. T. (1905), *Journal of Hygiene*, Vol. v. pp. 333—379.

CHART I

BOMBAY,

1897—1906

1897

1898

Jan. Feb. March April May June July Aug. Sept. Oct. Nov. Dec. Jan. Feb.

100 100° F.

+ 500

90 90° F.

+ 400

80 80° F.

+ 300

70 70° F.

+ 200

60 60° F.

+ 100

50 50° F.

0

40 40° F.

- 100

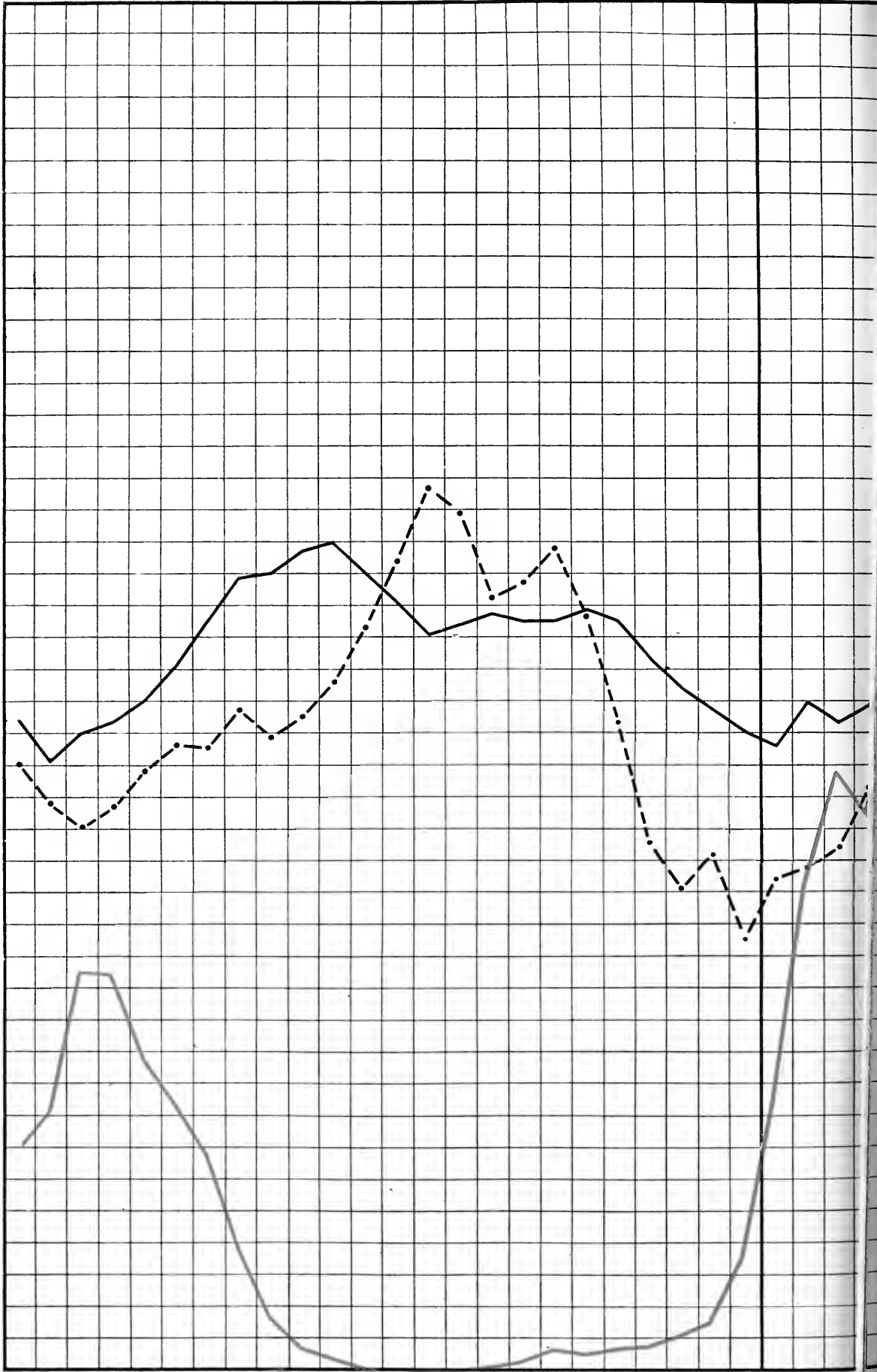
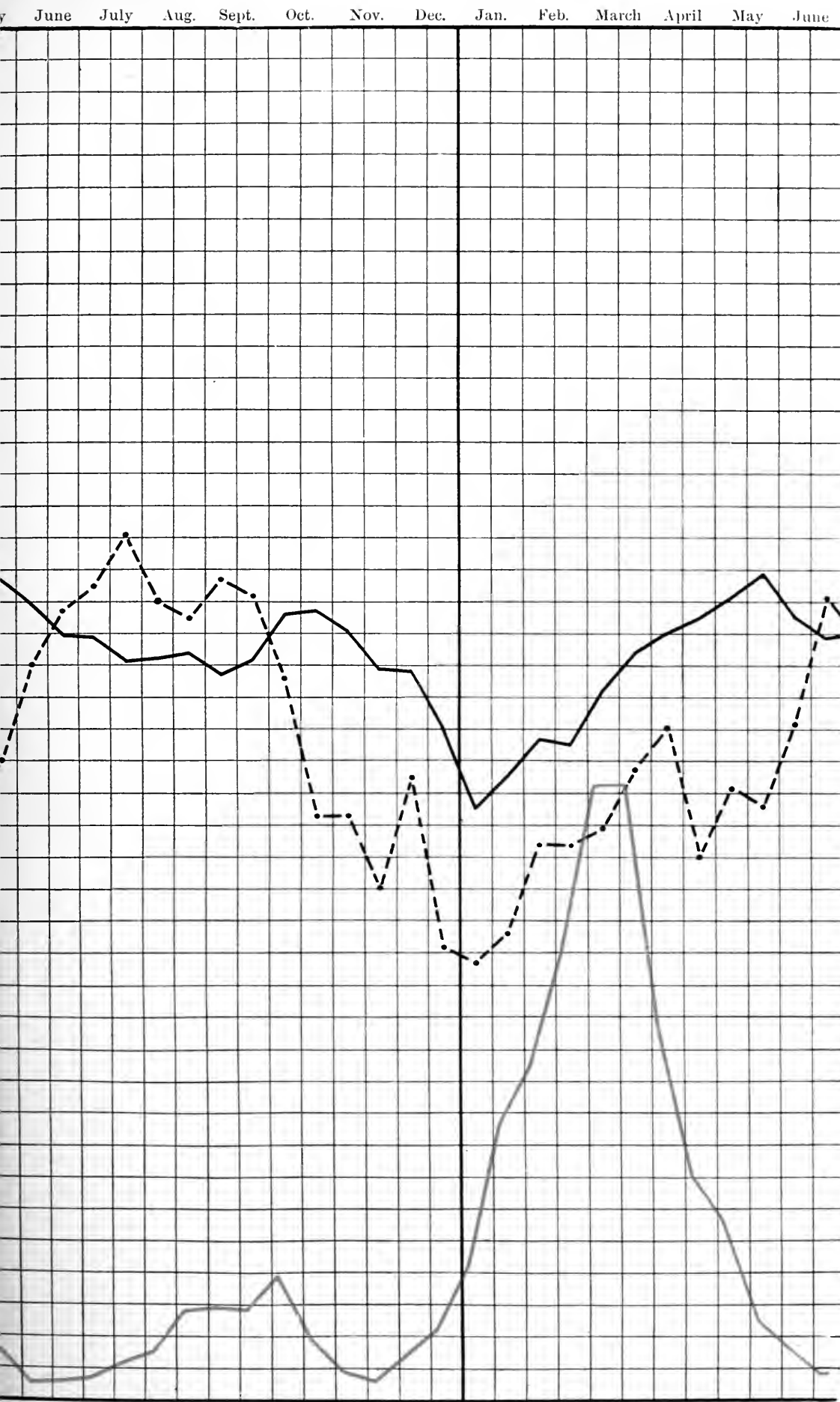


CHART I

1899



BOMBAY, 1897—1906

- Plague deaths
- Mean temperature
- Humidity

CHART I (continued)

BOMBAY,

1897 — 1906

1900

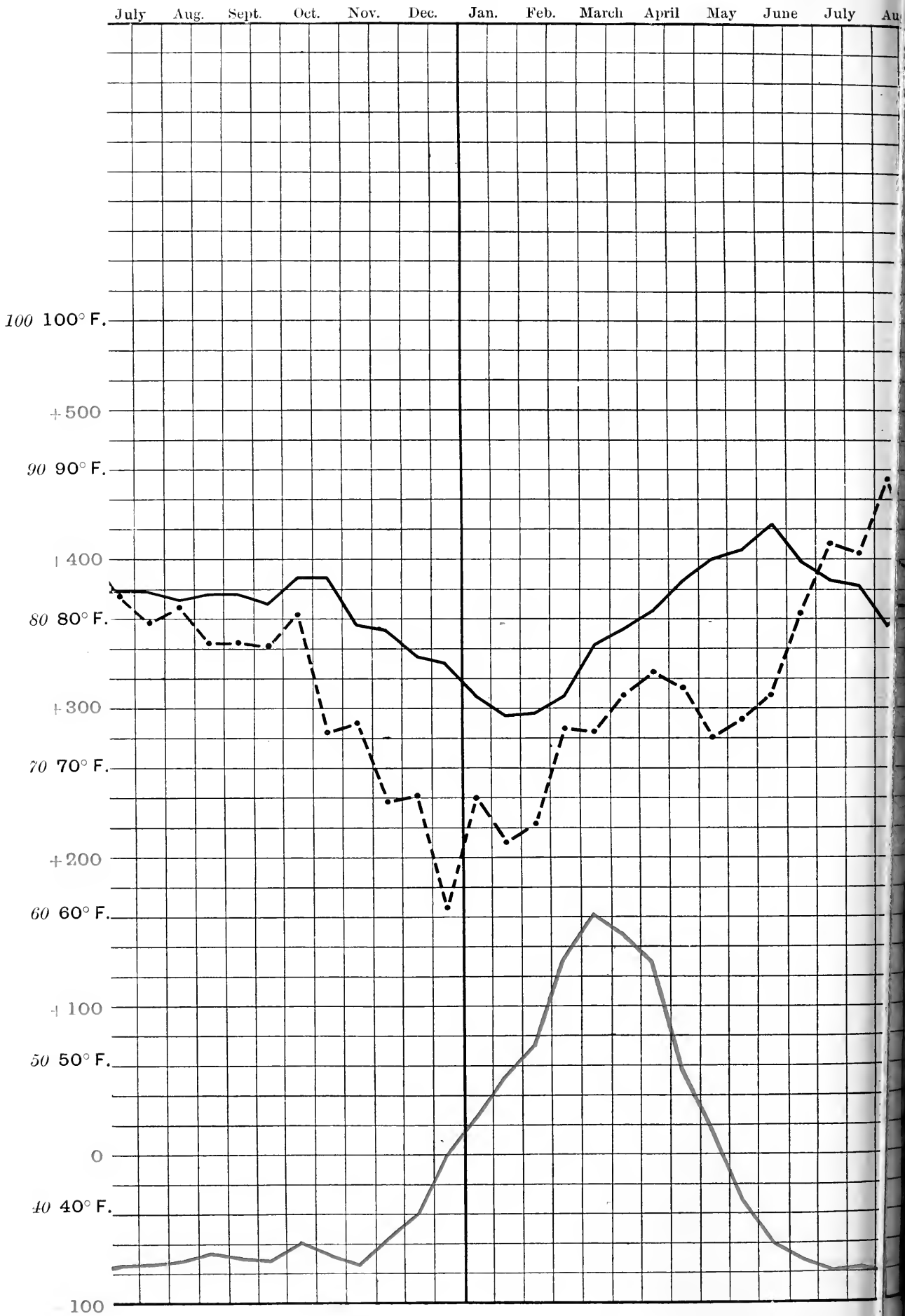
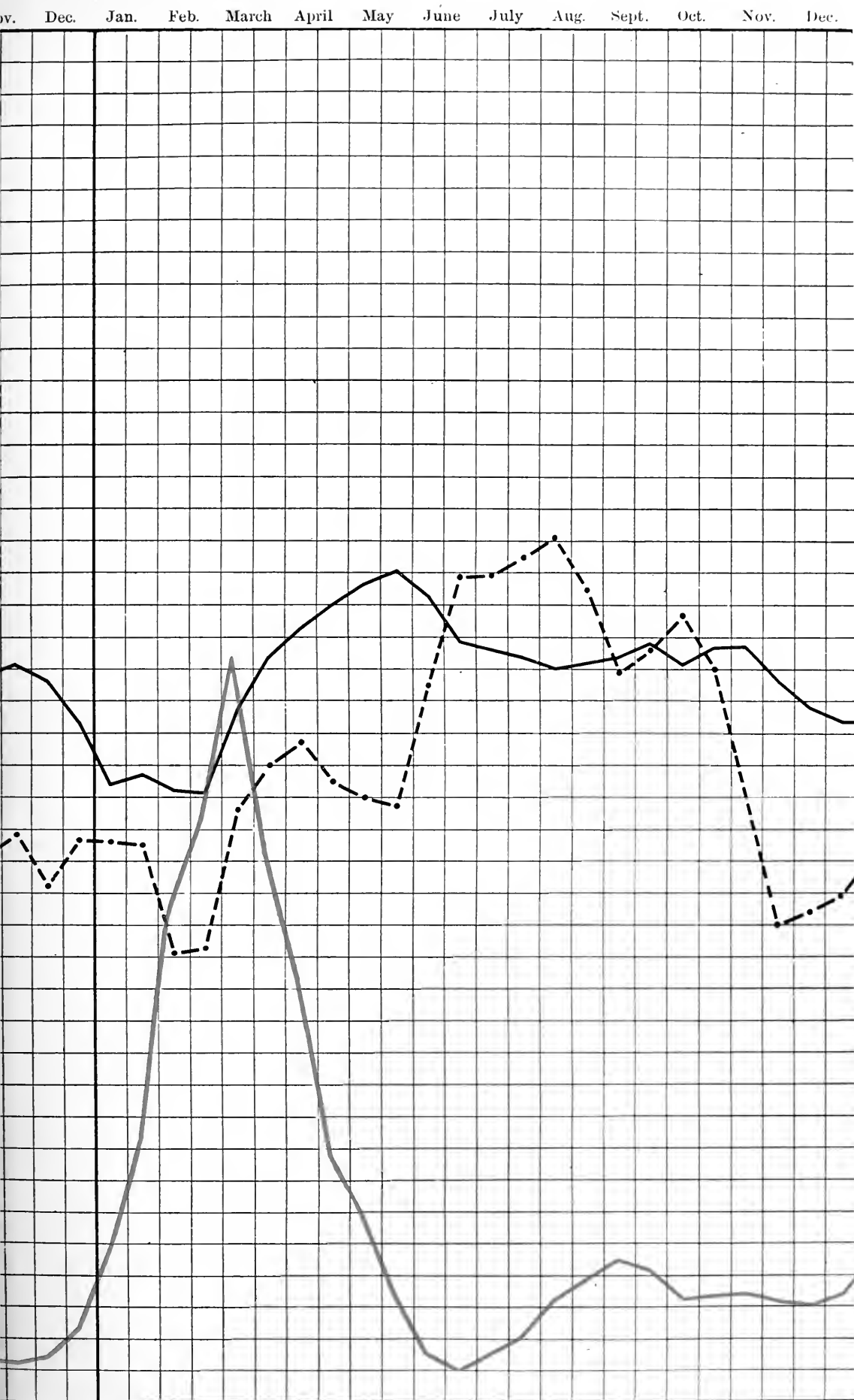


CHART I (continued)

1901



BOMBAY, 1897—1906

- Plague deaths
- Mean temperature
- Humidity

CHART I (continued)

BOMBAY,

1897—1906

1902

1903

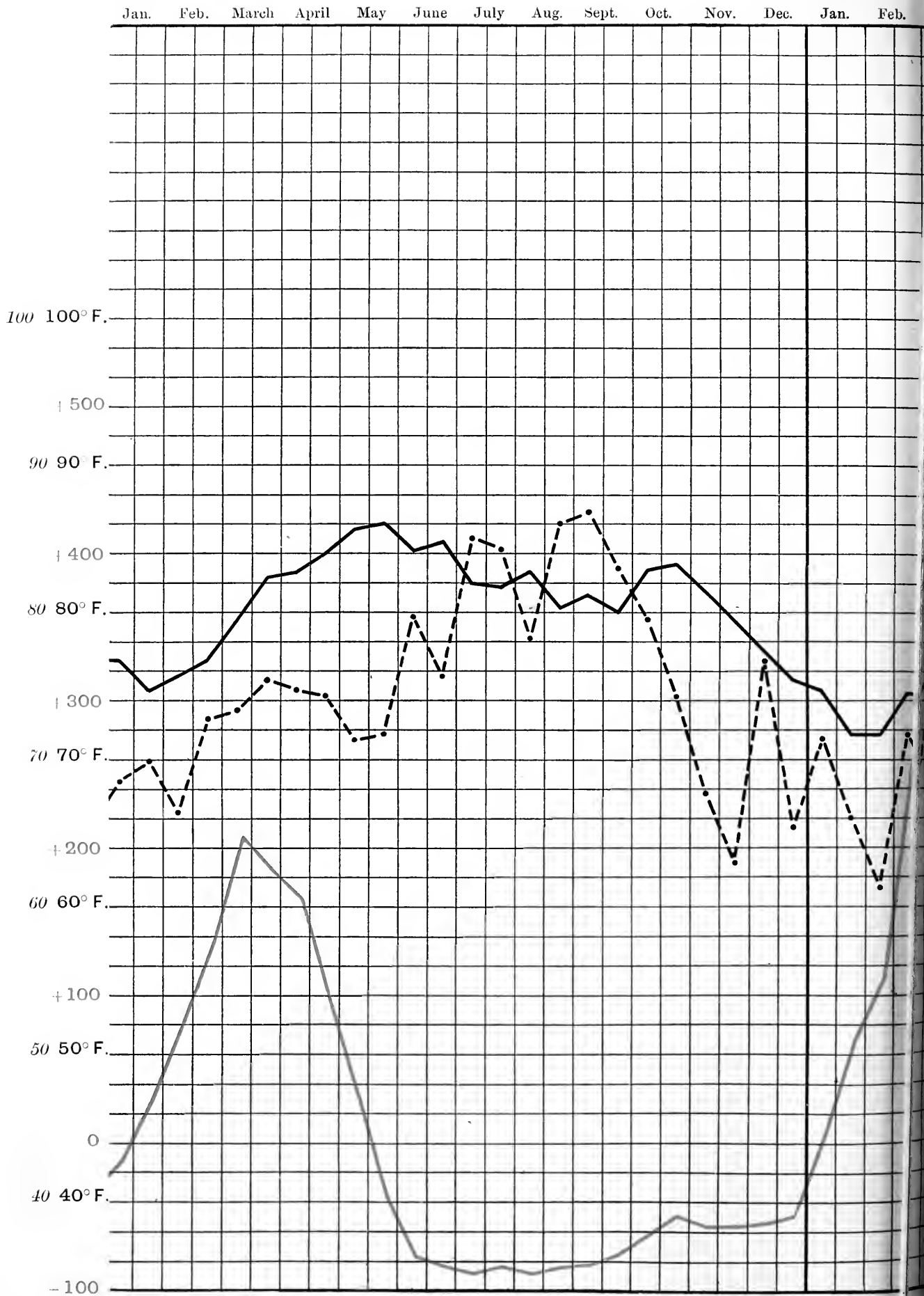
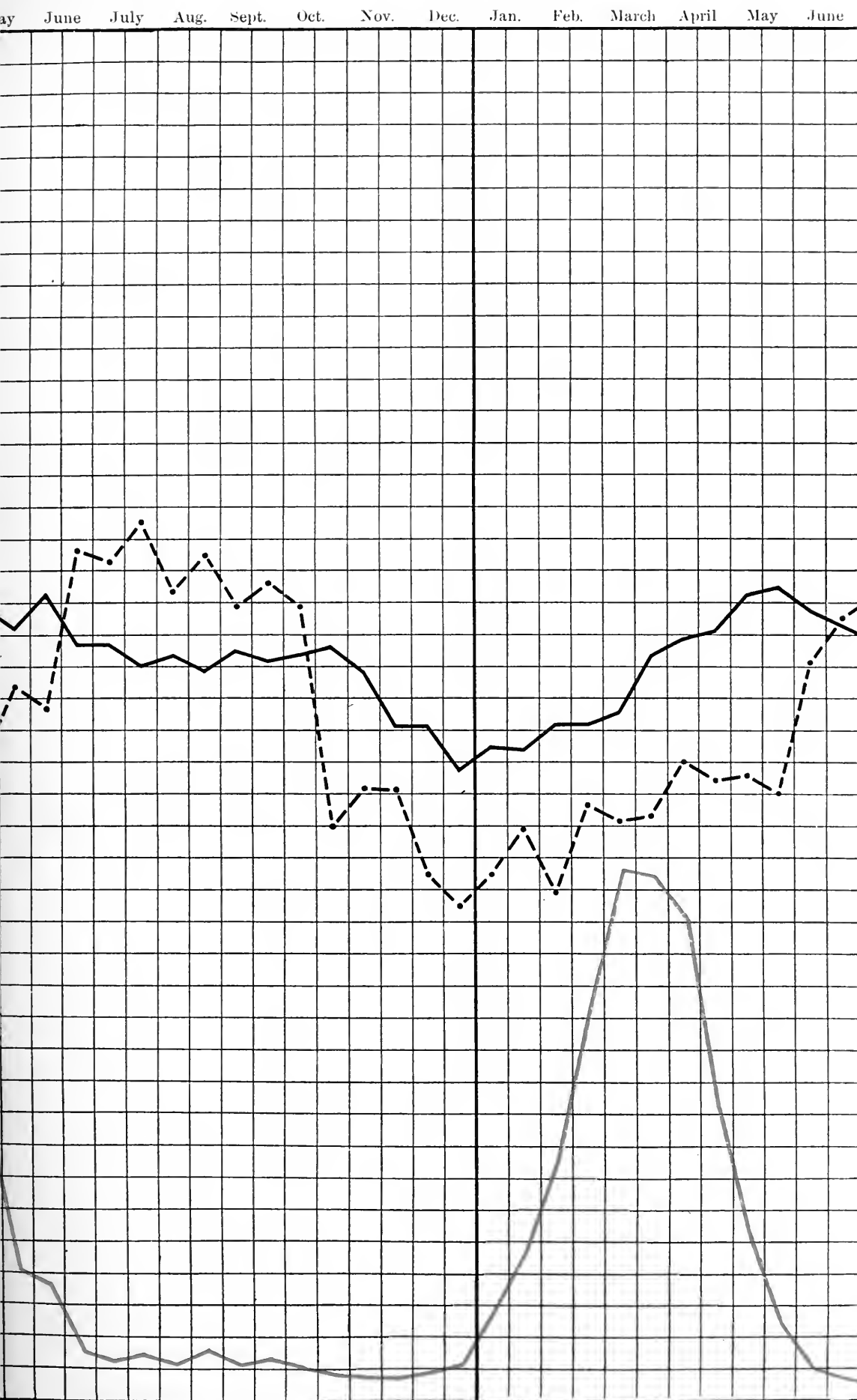


CHART I (continued)

1904



BOMBAY, 1897—1906

- Plague deaths
- Mean temperature
- Humidity

CHART I (continued)

BOMBAY,

1897—1906

1905

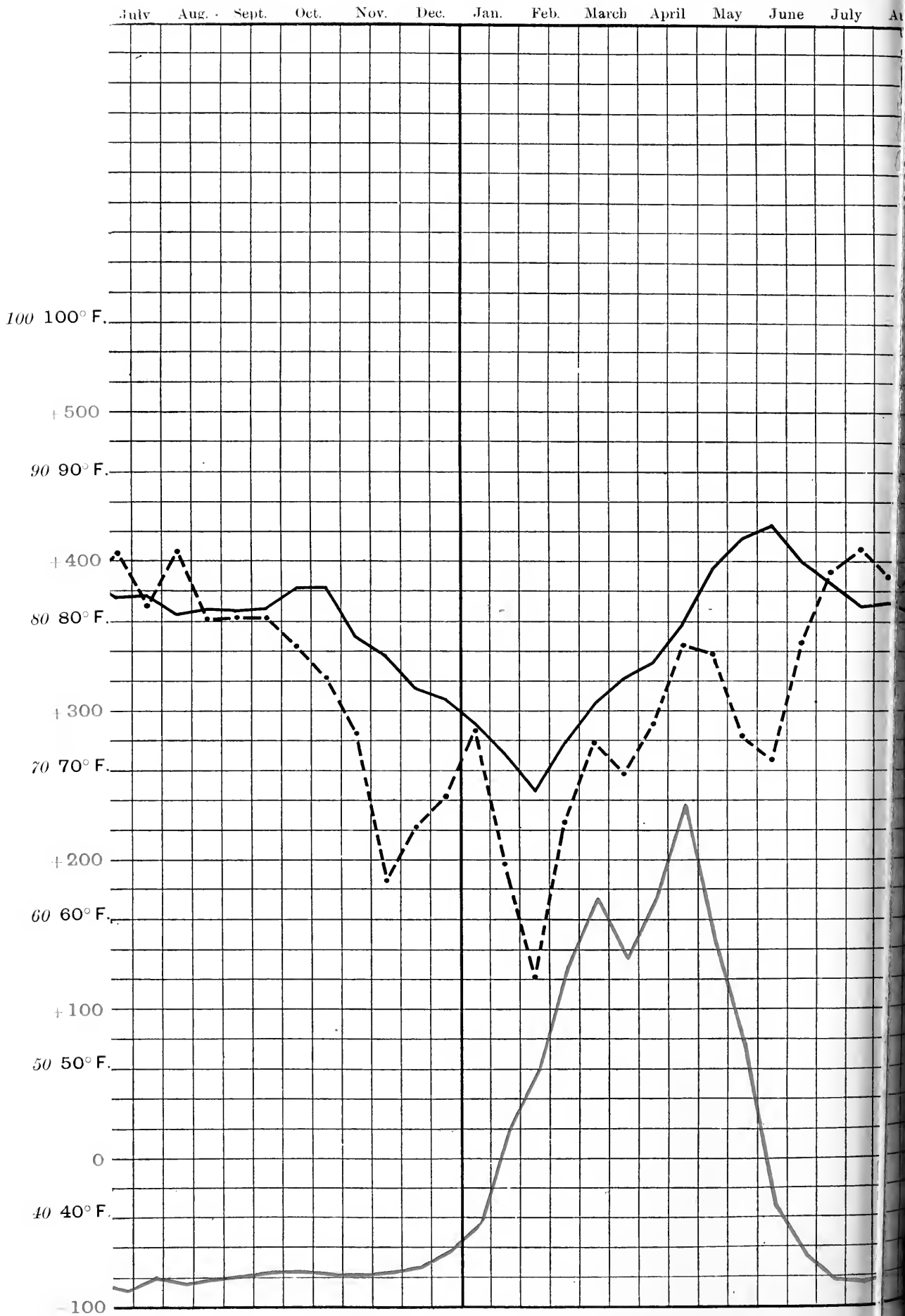
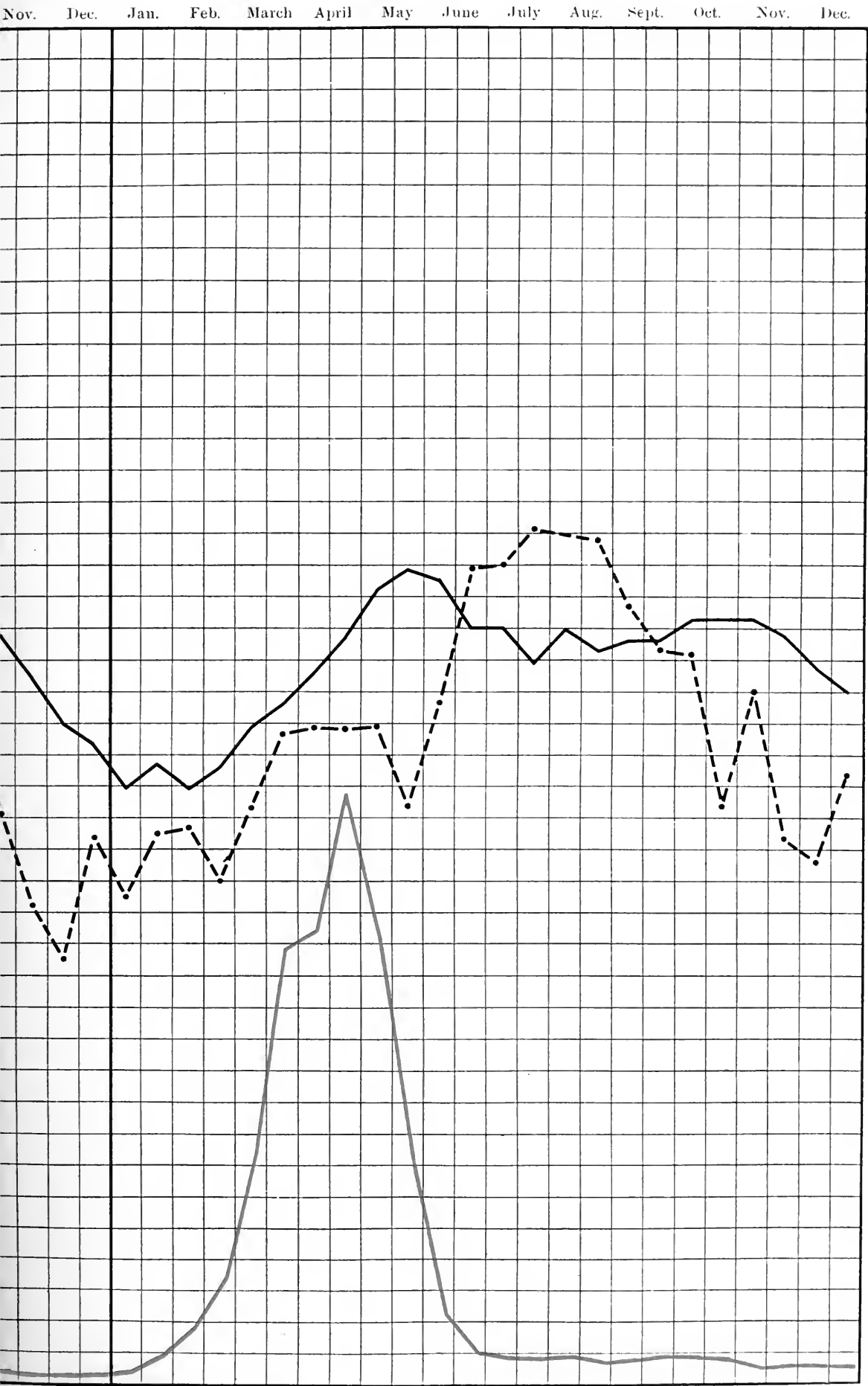


CHART I (continued)

1906



BOMBAY, 1897—1906

- Plague deaths
- Mean temperature
- Humidity

CHART II

POONA, CITY AND CANTONMENT,

1897—1906

1897

1898

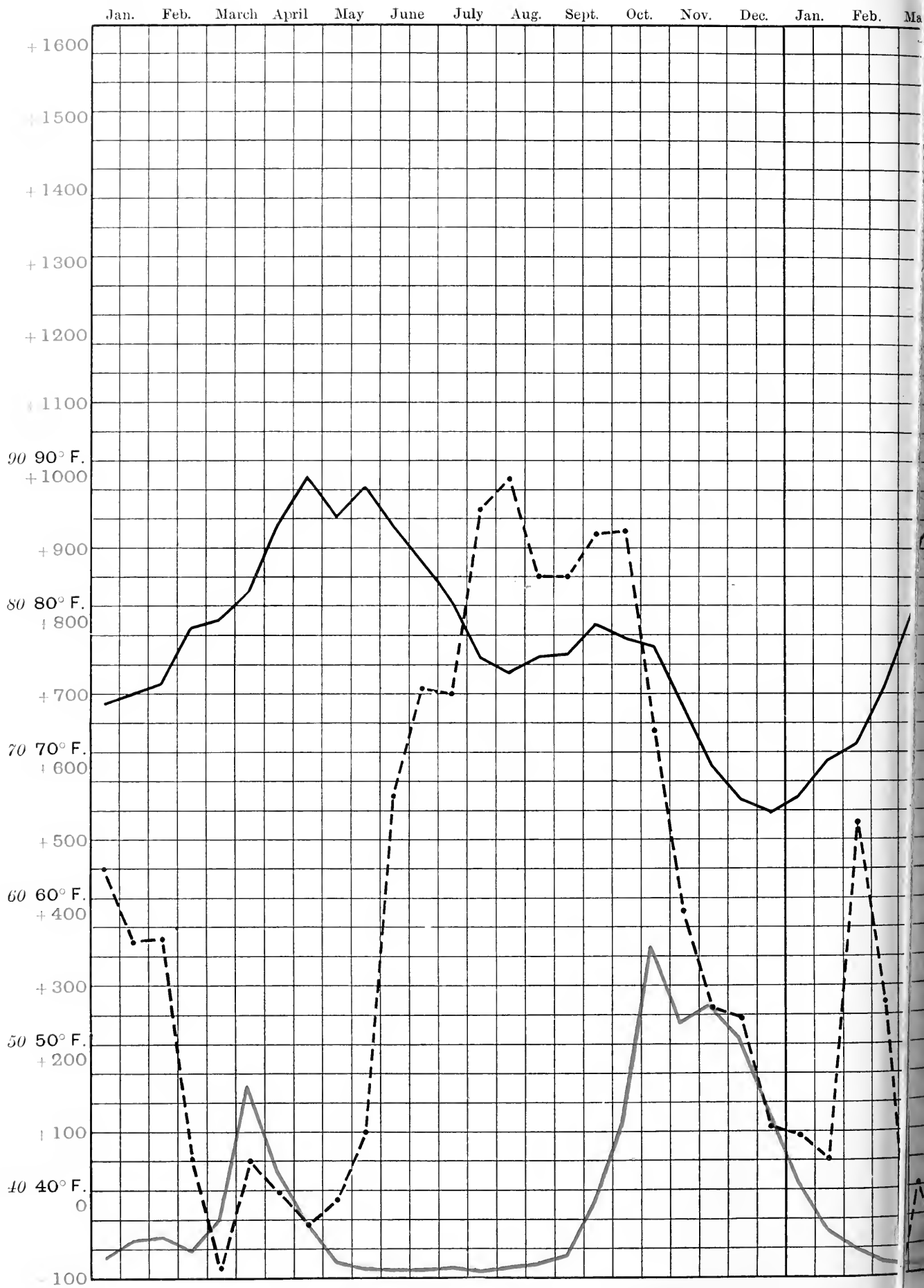
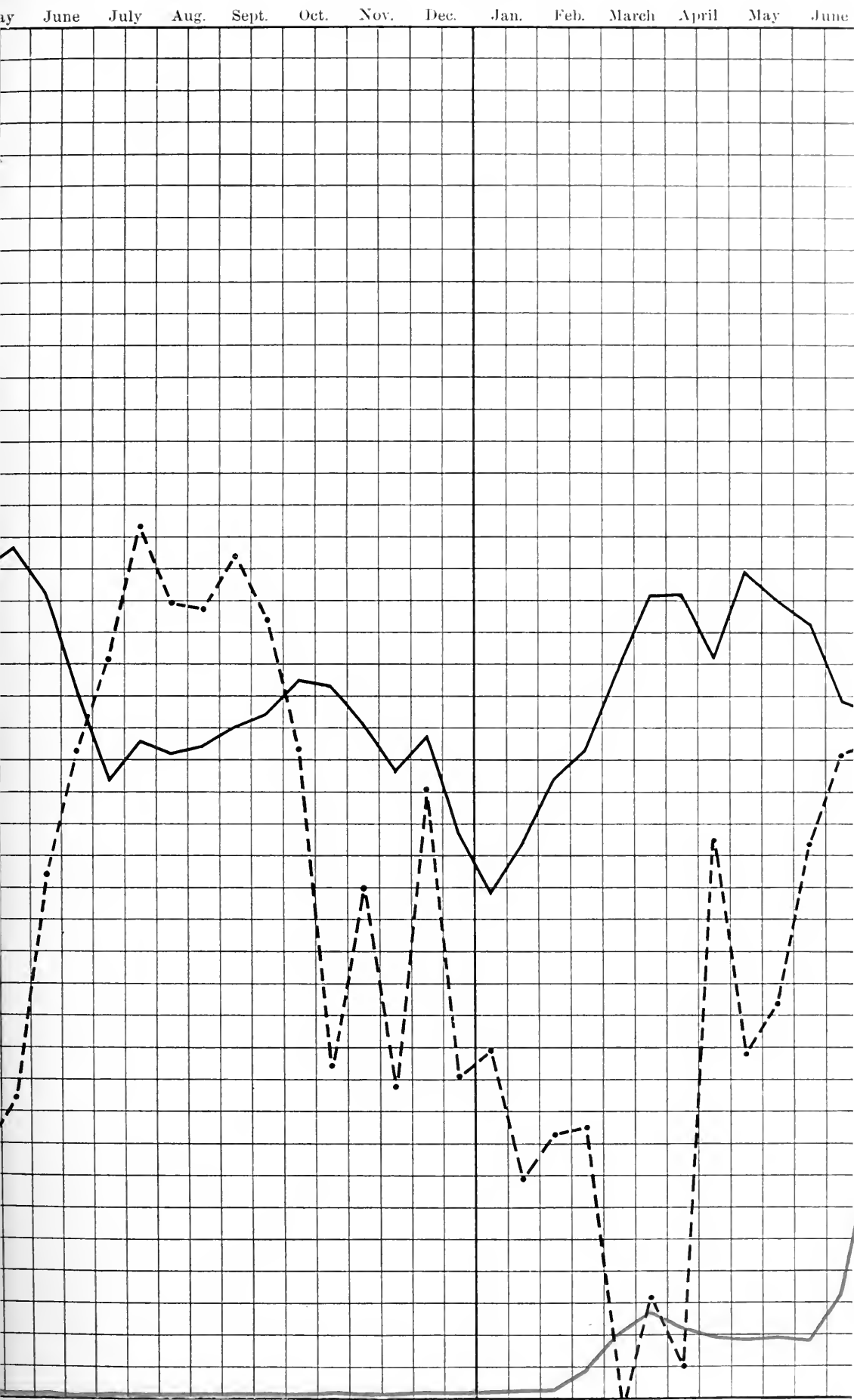


CHART II

1899



POONA, CITY AND CANTONMENT, 1897—1906

- Plague deaths
- - - Mean temperature
- Humidity

CHART II (continued)

POONA, CITY AND CANTONMENT,

1897—1906

1900

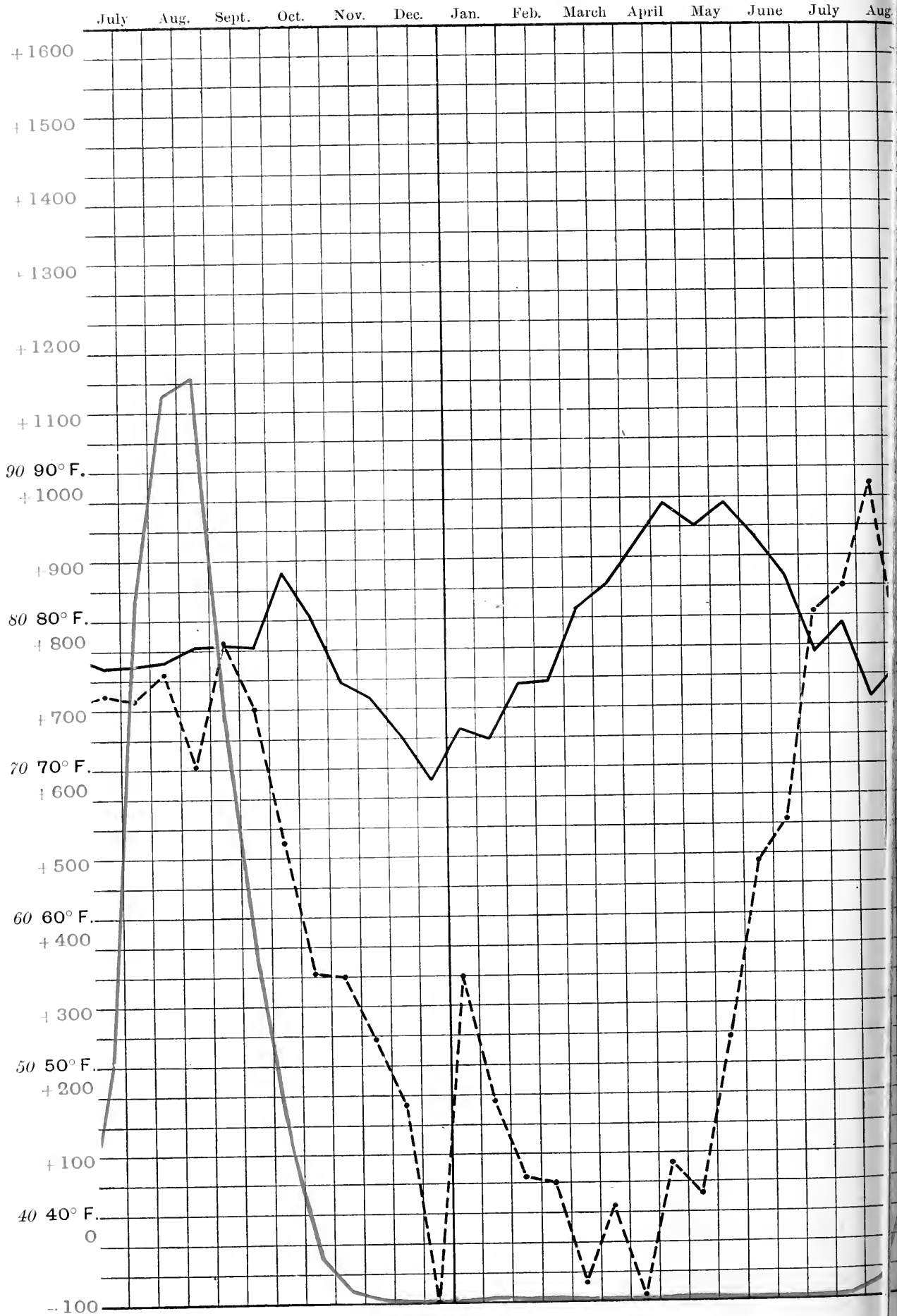
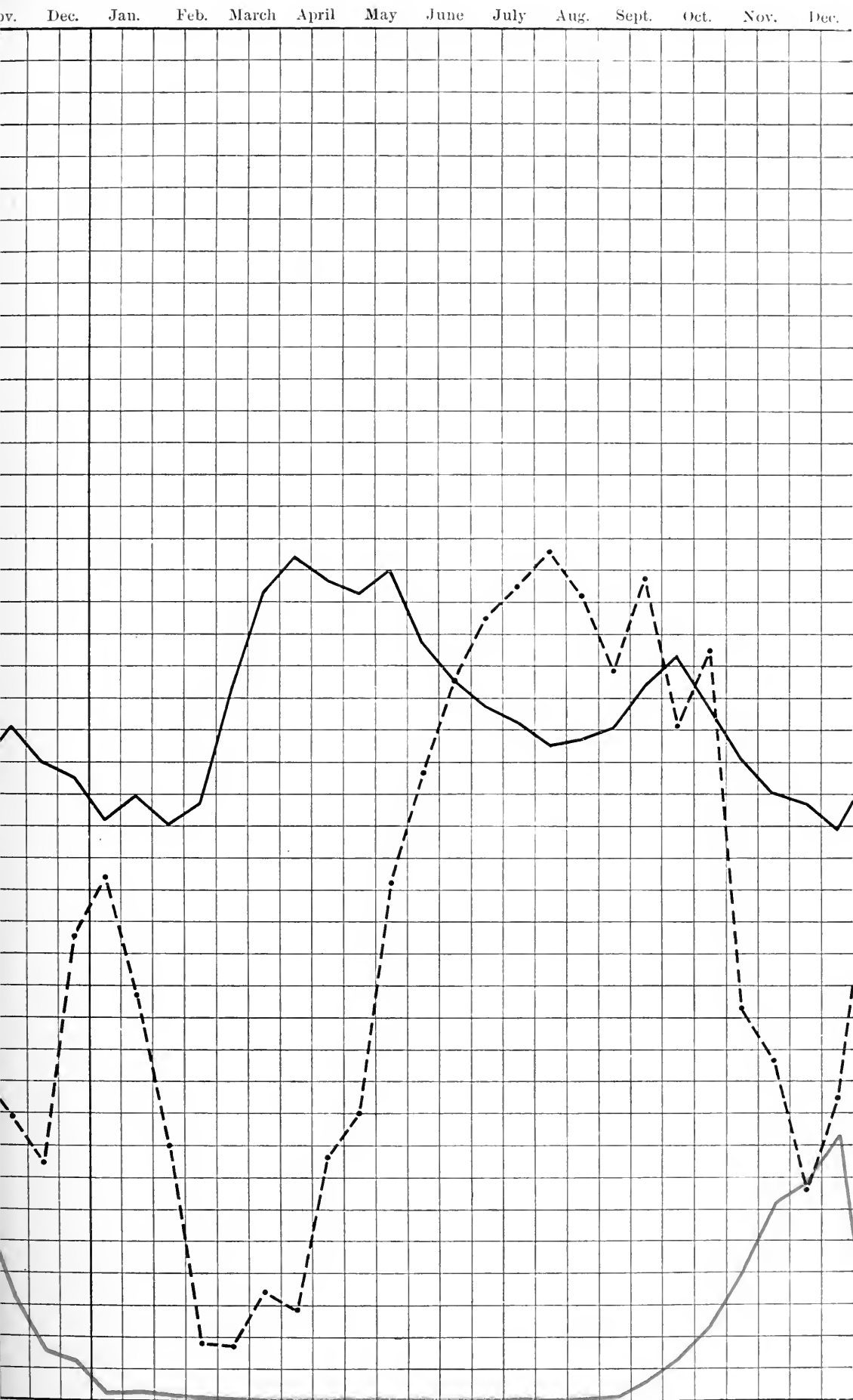


CHART II (continued)

1901



POONA, CITY AND CANTONMENT, 1897—1906

- Plague deaths
- Mean temperature
- Humidity



CHART II (continued)

POONA, CITY AND CANTONMENT,

1897—1906

1902

1903

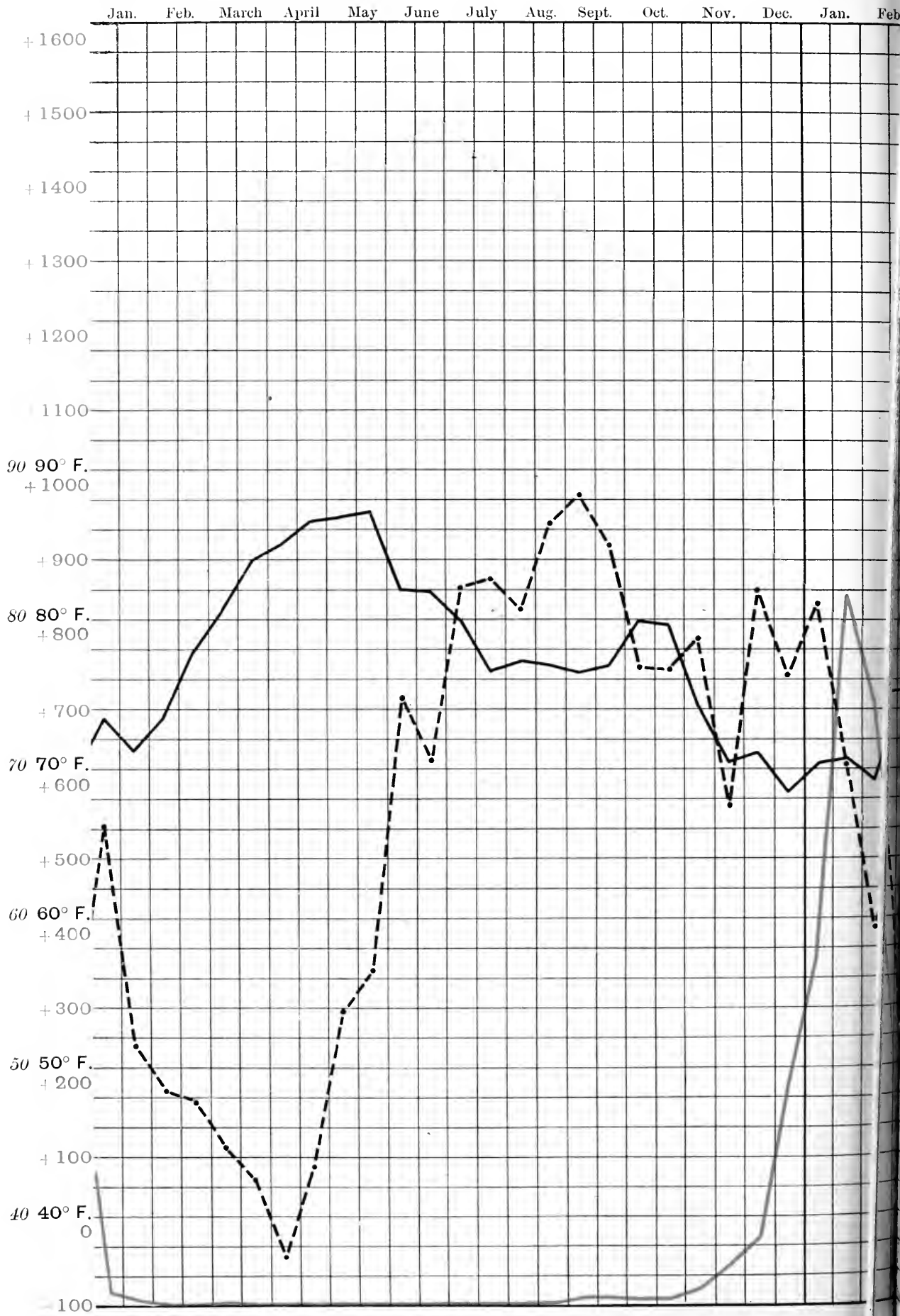
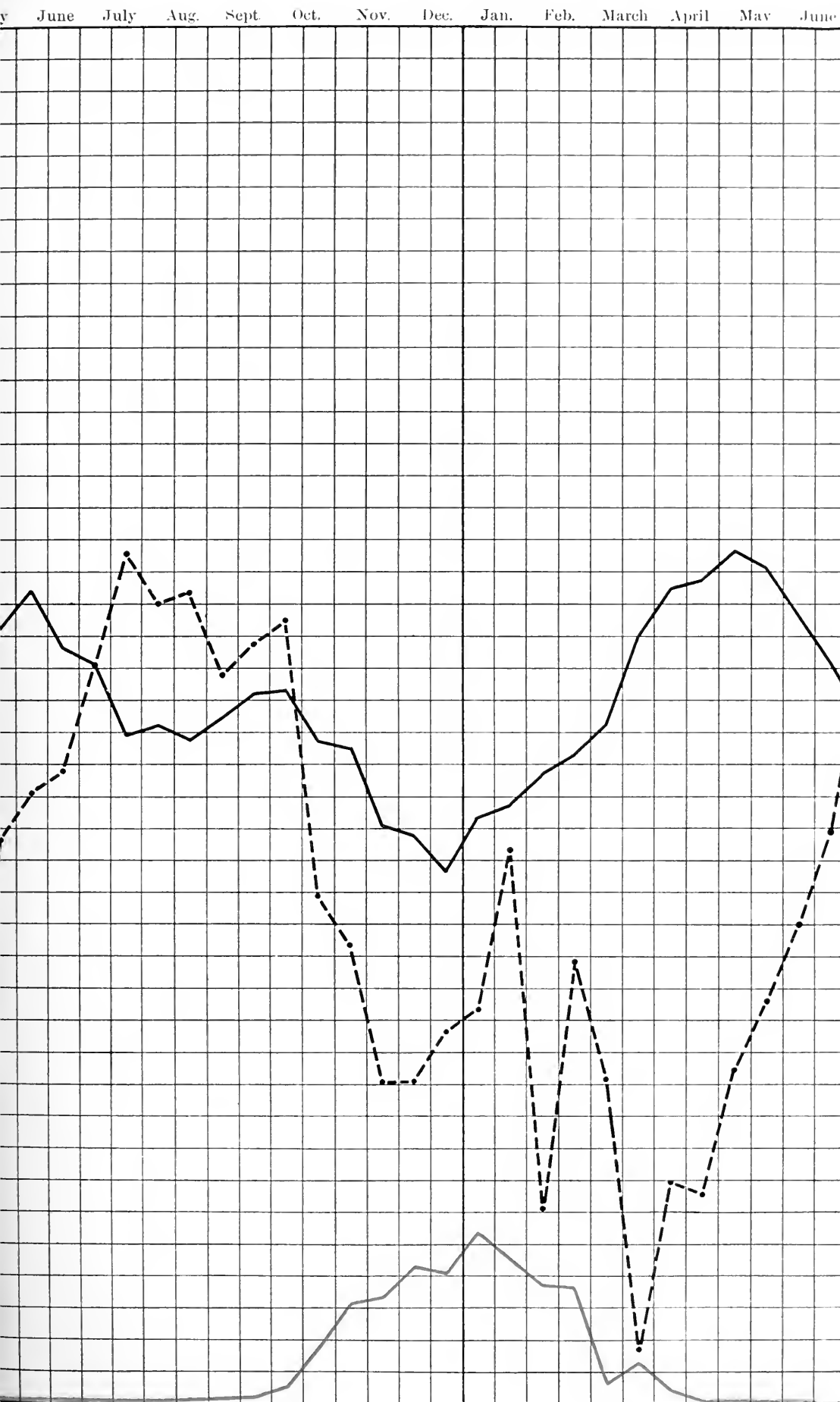


CHART II (continued)

1904



BOONA, CITY AND CANTONMENT, 1897—1906

- Plague deaths
- Mean temperature
- Humidity

CHART II (continued)

POONA, CITY AND CANTONMENT,

1897—1906

1905

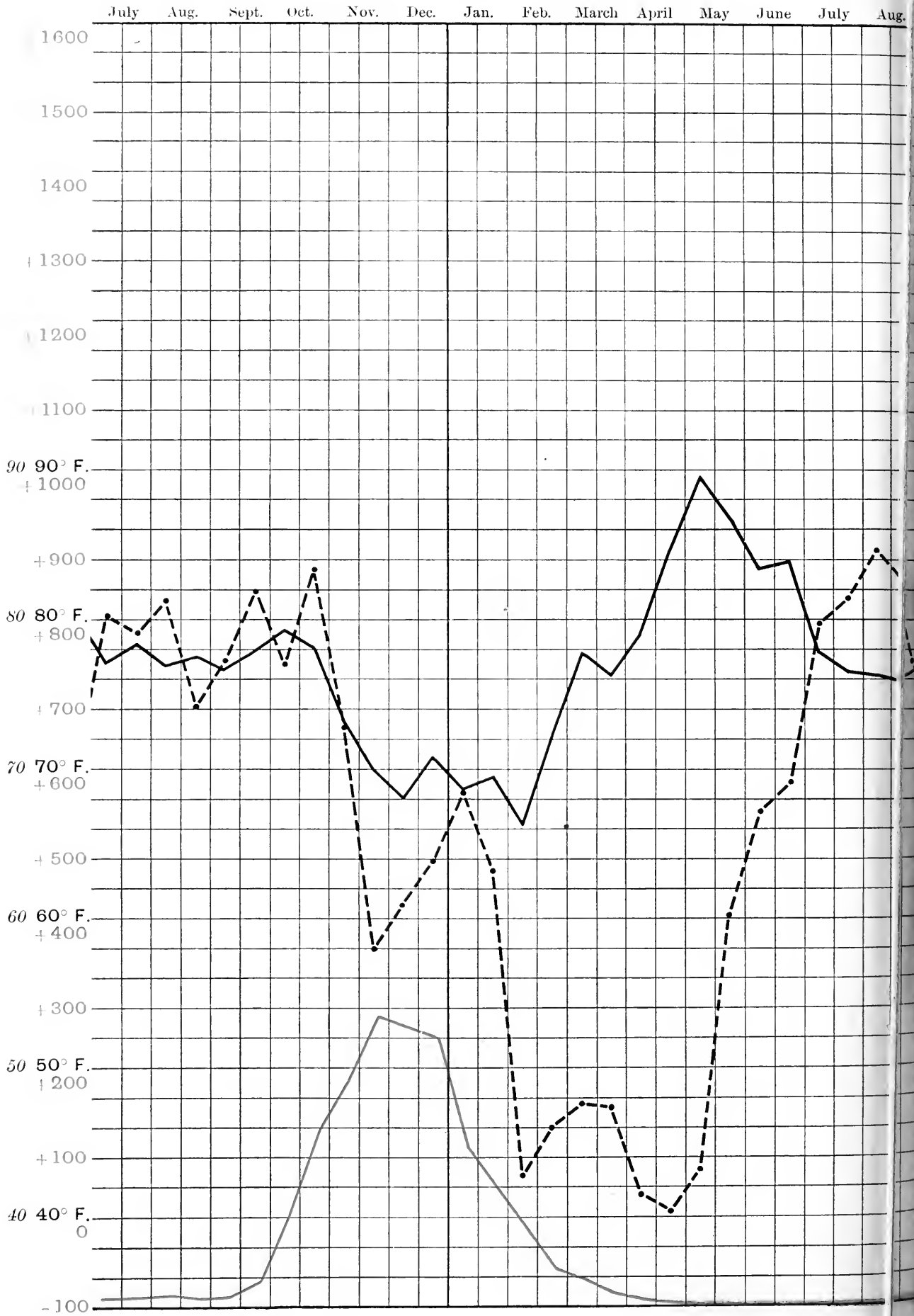
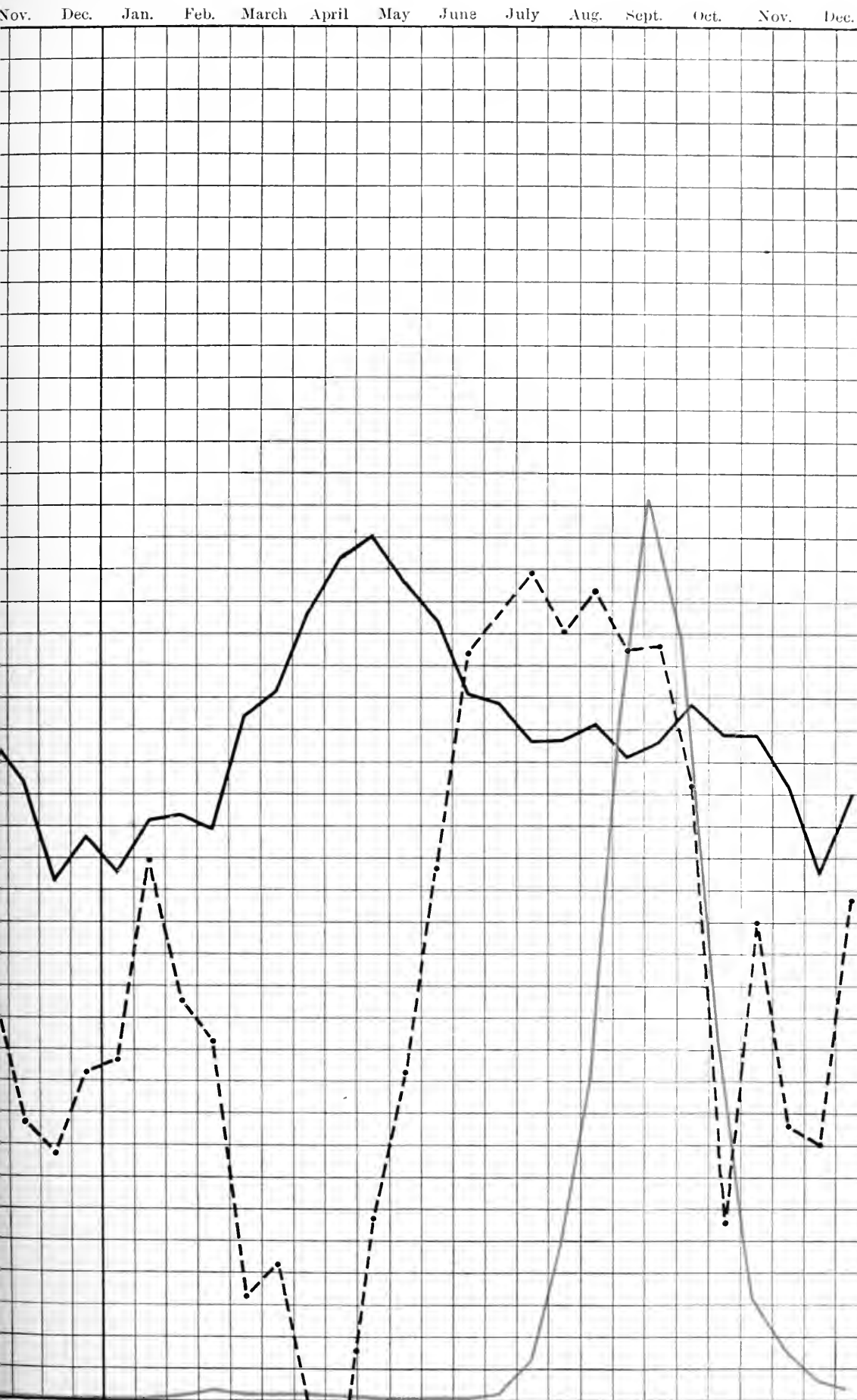


CHART II (continued)

1906



POONA, CITY AND CANTONMENT, 1897—1906

- Plague deaths
- Mean temperature
- Humidity

CHART III

NAGPUR,

1903—1906

1903

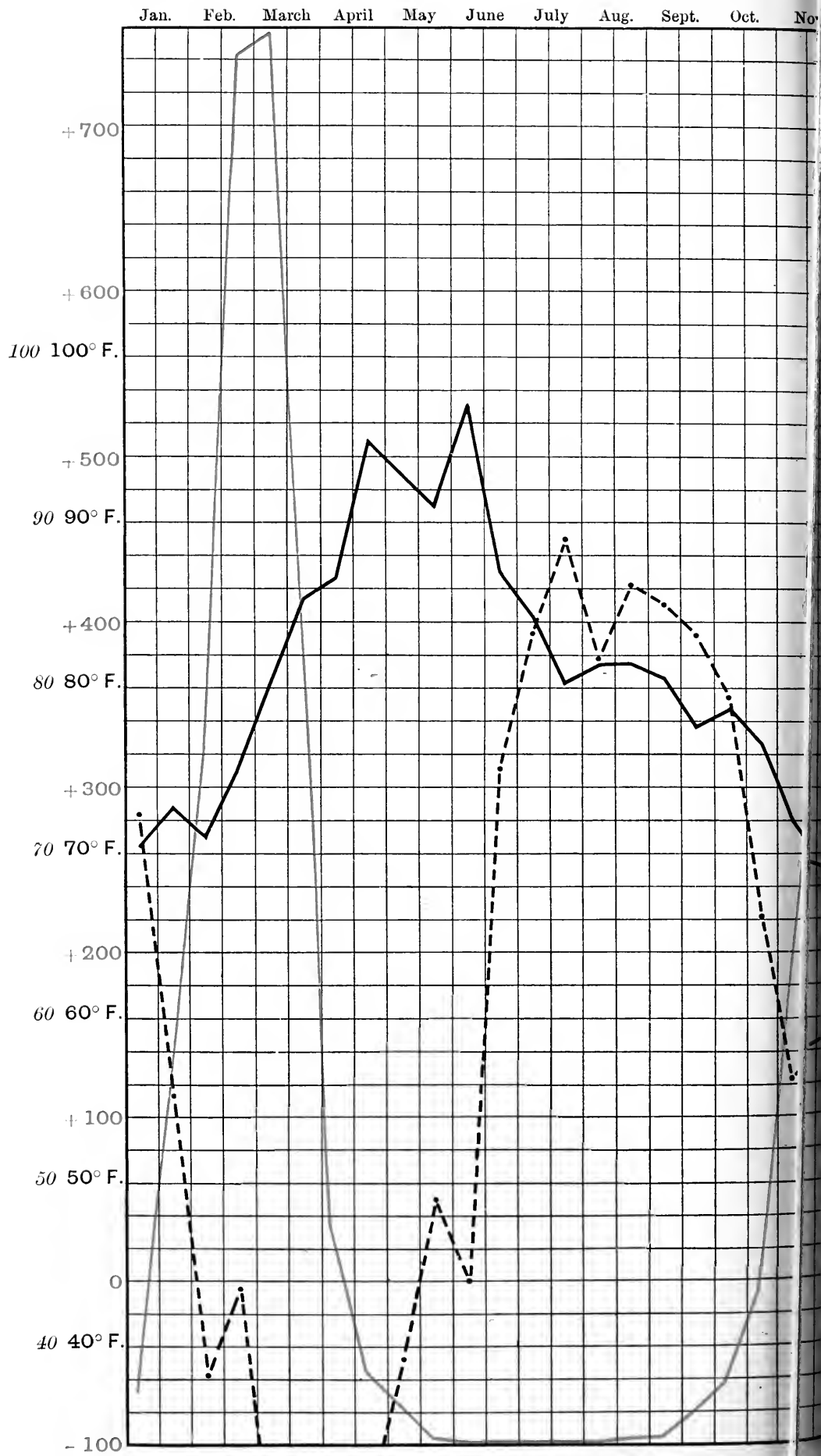
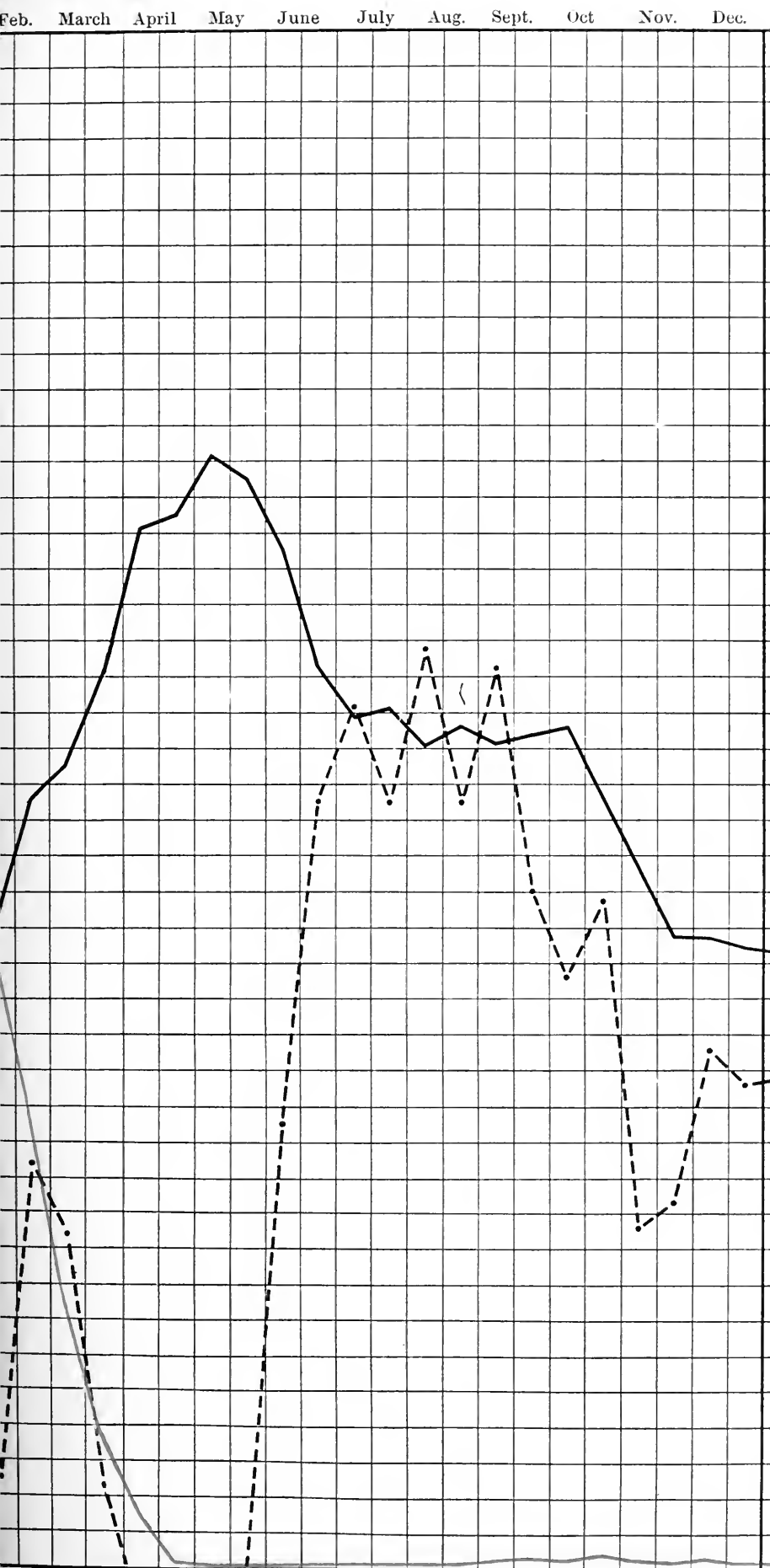


CHART III



NAGPUR, 1903—1906

- Plague deaths
- Mean temperature
- Humidity

CHART III (continued)

NAGPUR,

1903—1906

1905

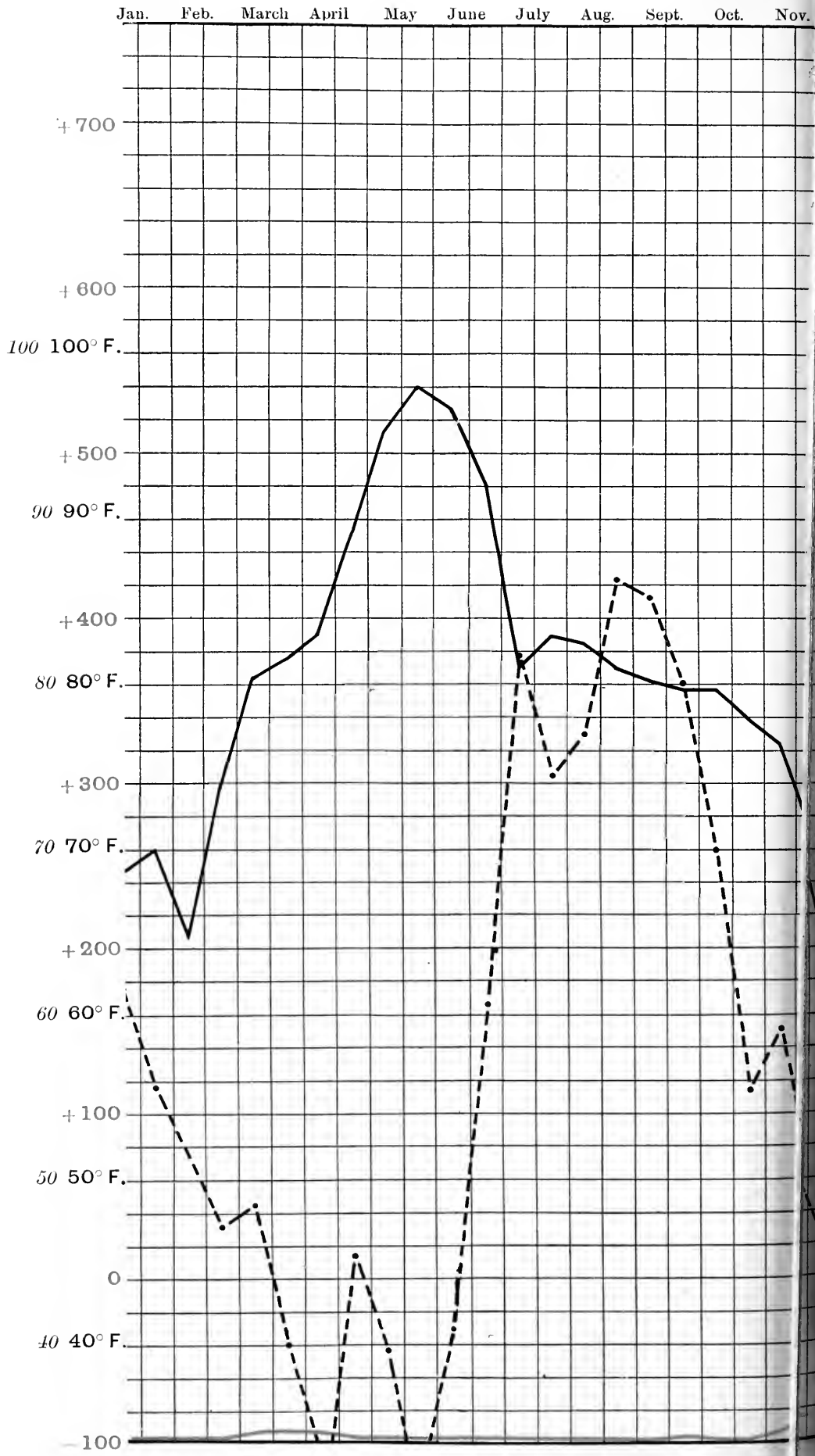
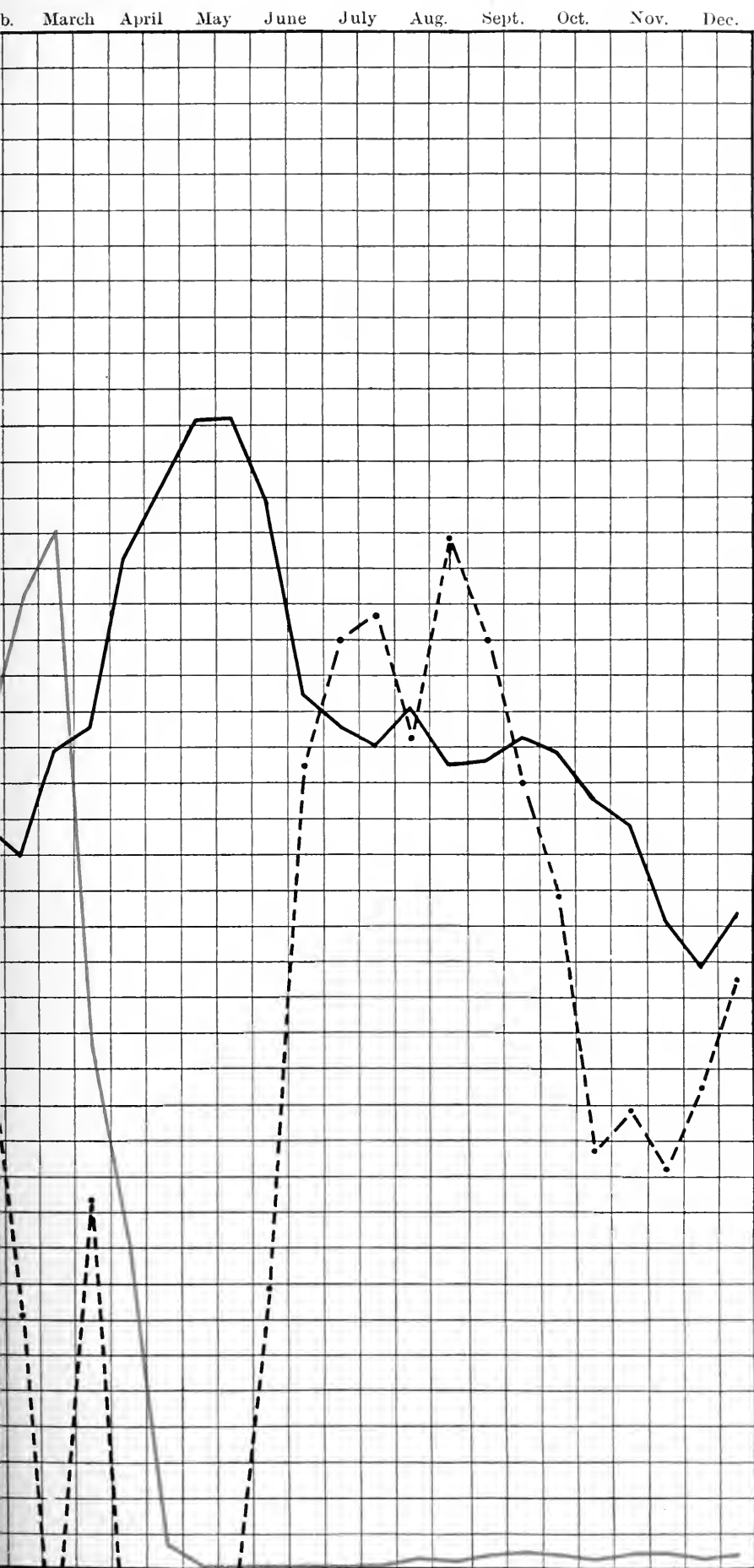


CHART III (continued)



NAGPUR, 1903—1906

- Plague deaths
- - - Mean temperature
- Humidity

CHART IV

BELGAUM,

1897 - 1906

1897

189

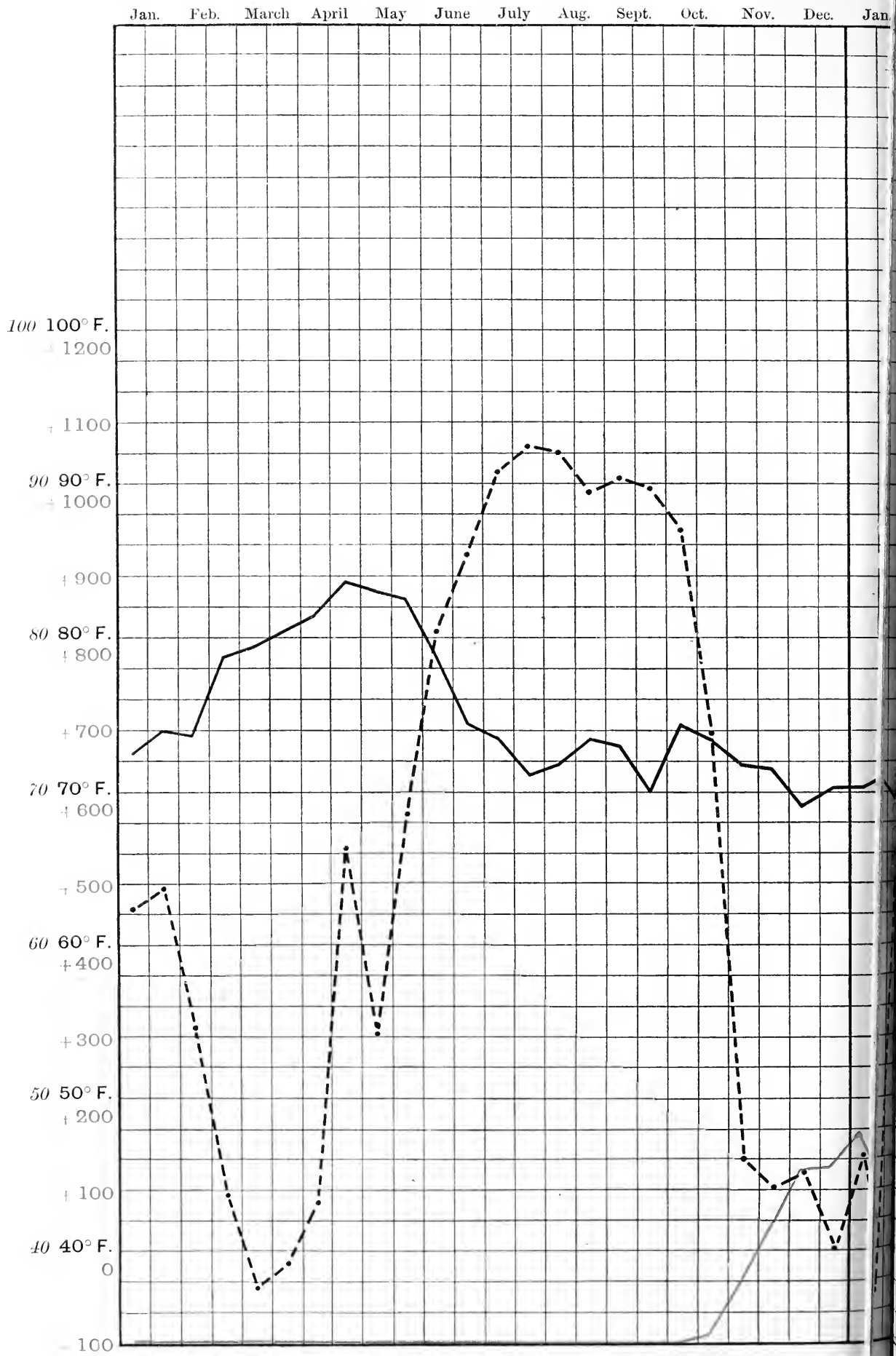
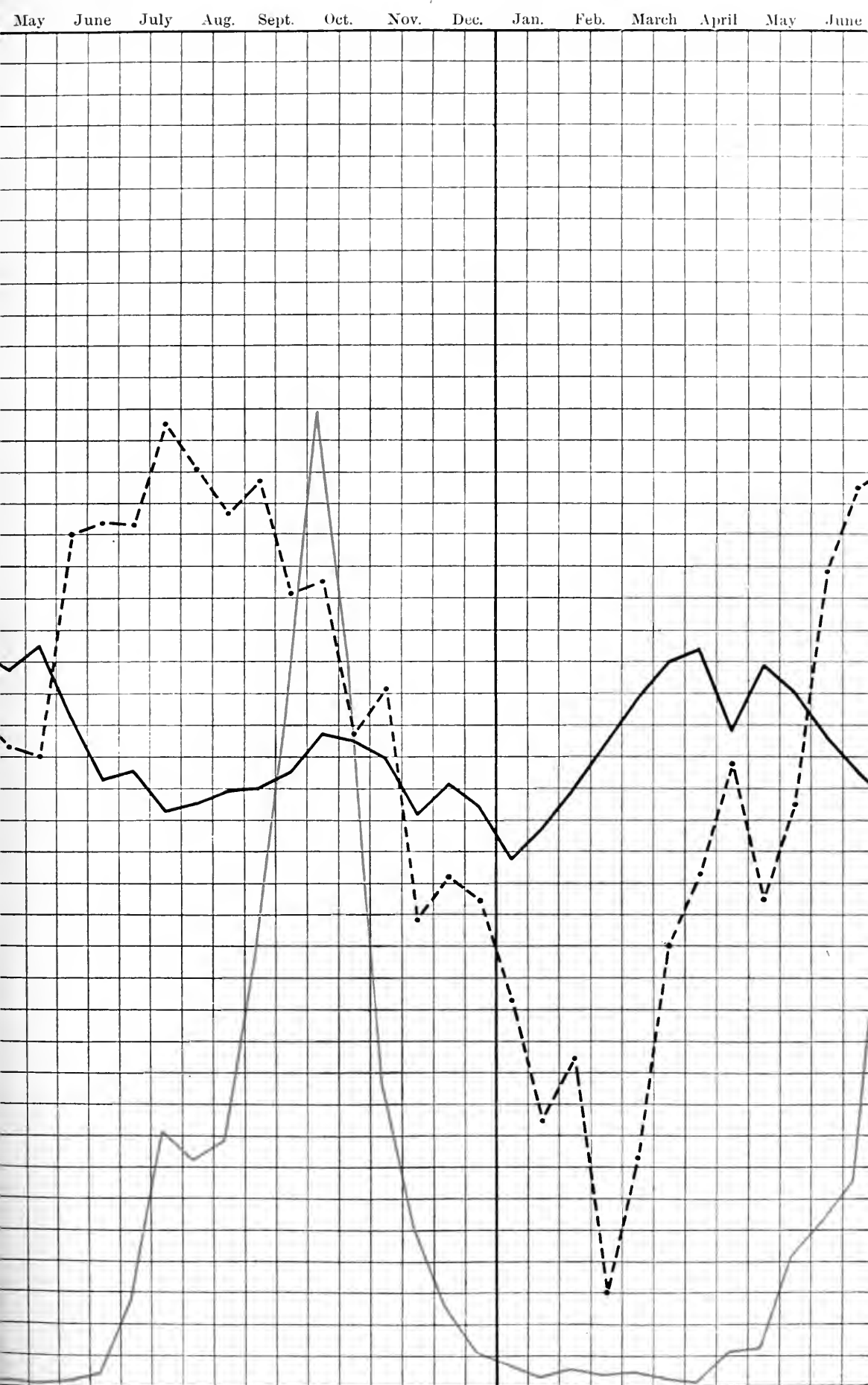


CHART IV

1899



BELGAUM, 1897—1906

- Plague deaths
- Mean temperature
- Humidity

CHART IV (continued)

BELGAUM,

1897—1906

1900

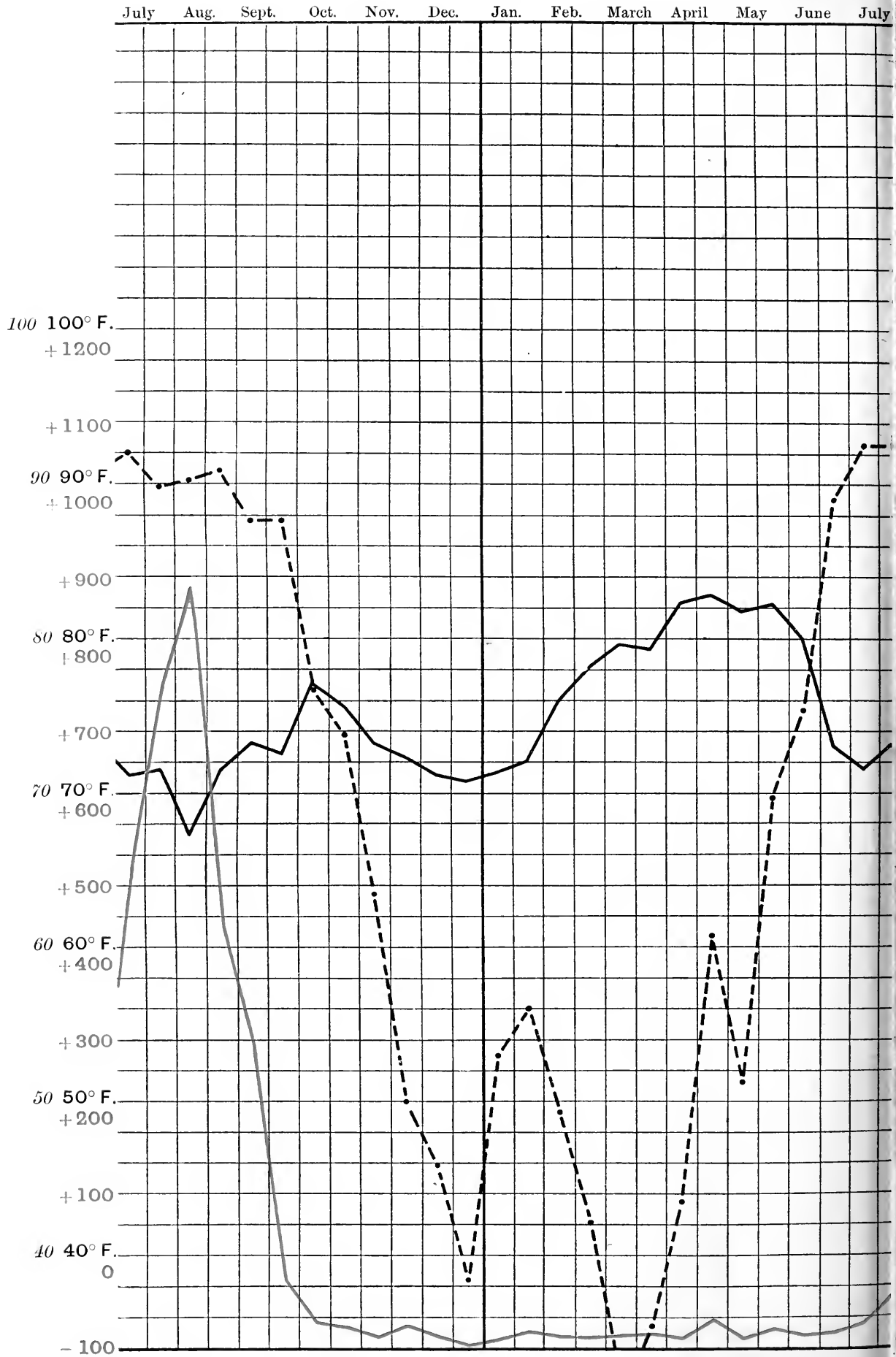
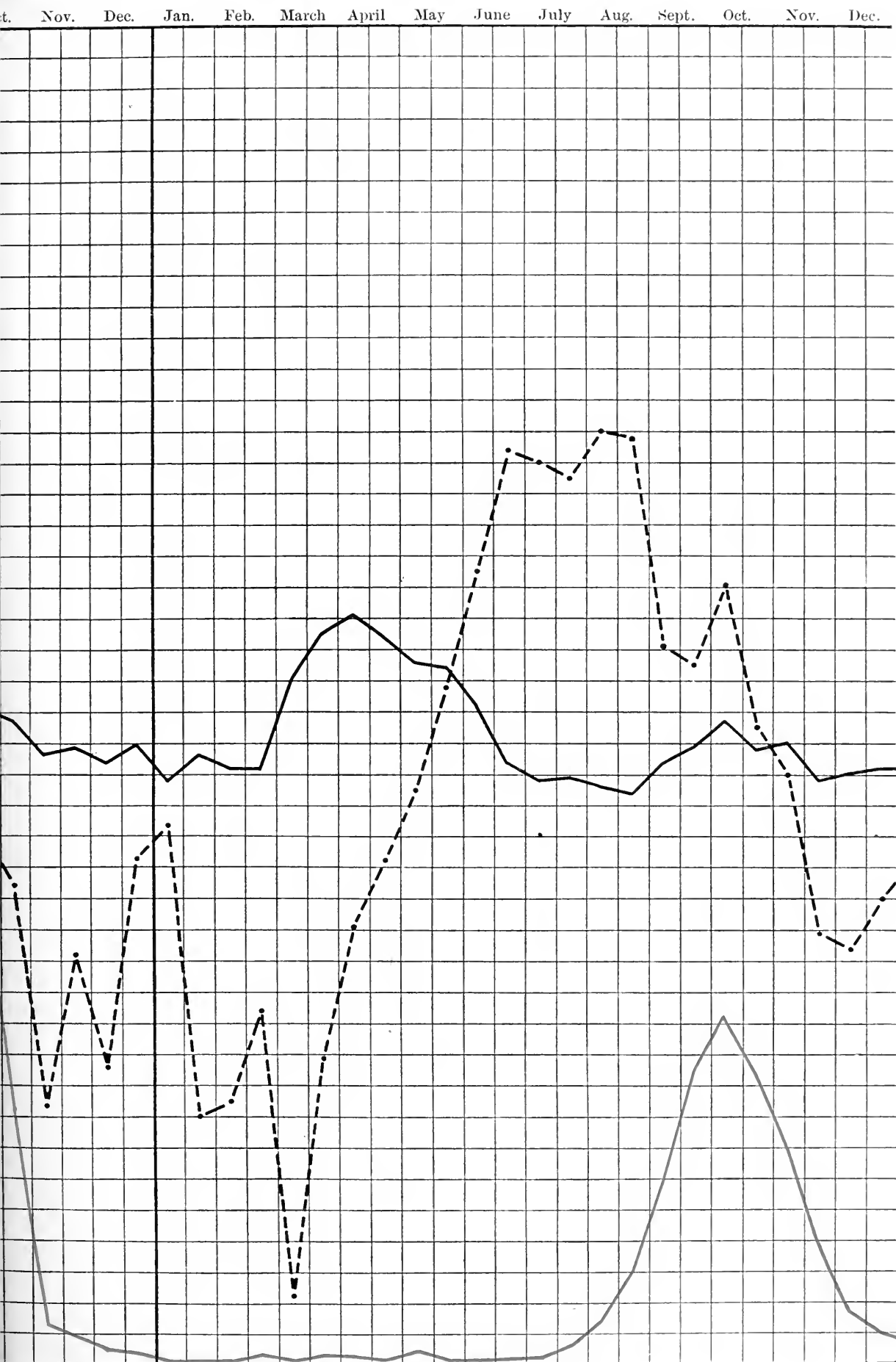


CHART IV (continued)

1901



BELGAUM, 1897—1906

- Plague deaths
- - - Mean temperature
- Humidity

CHART IV (continued)

BELGAUM,

1897—1906

1902

1903

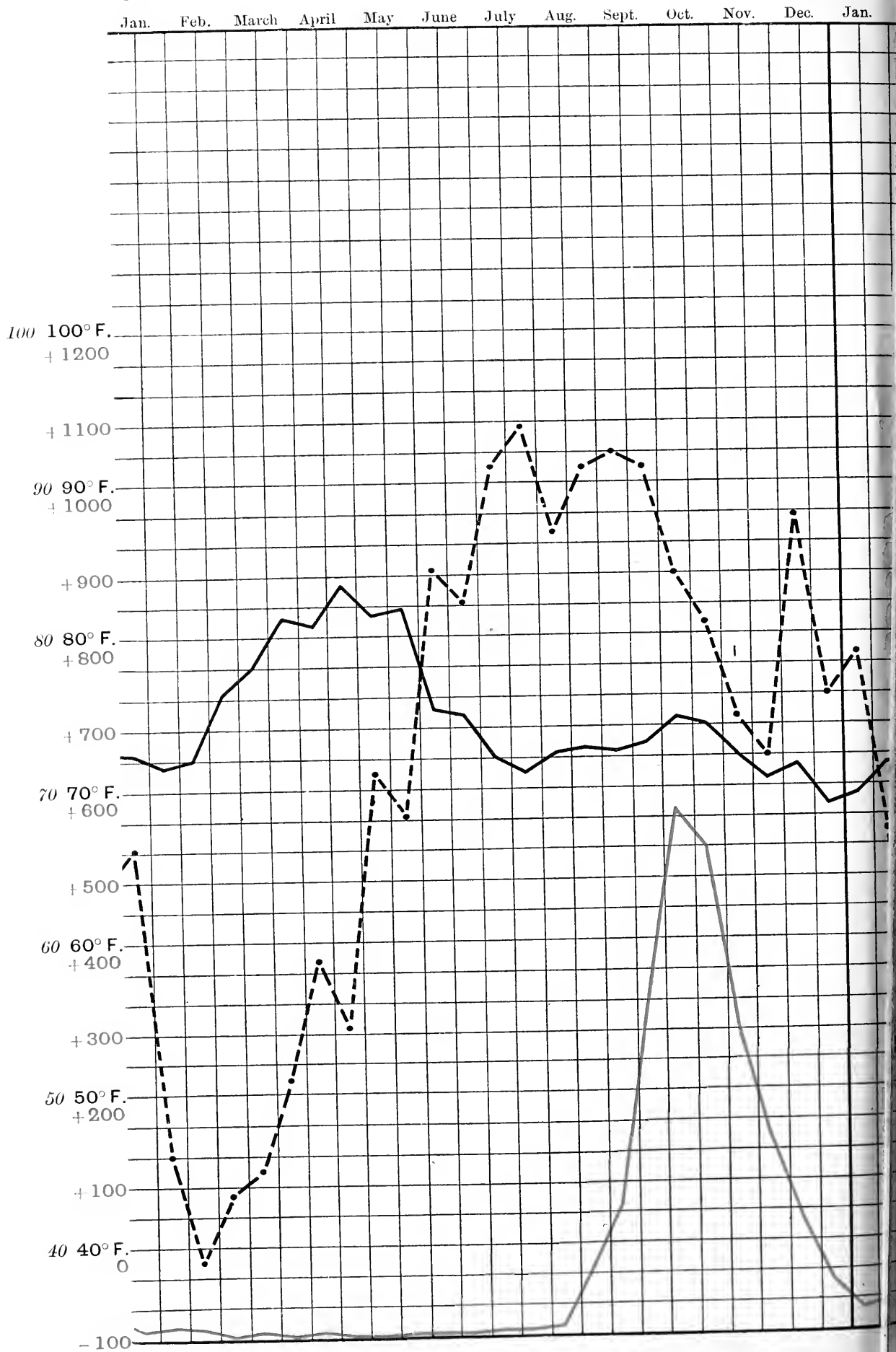
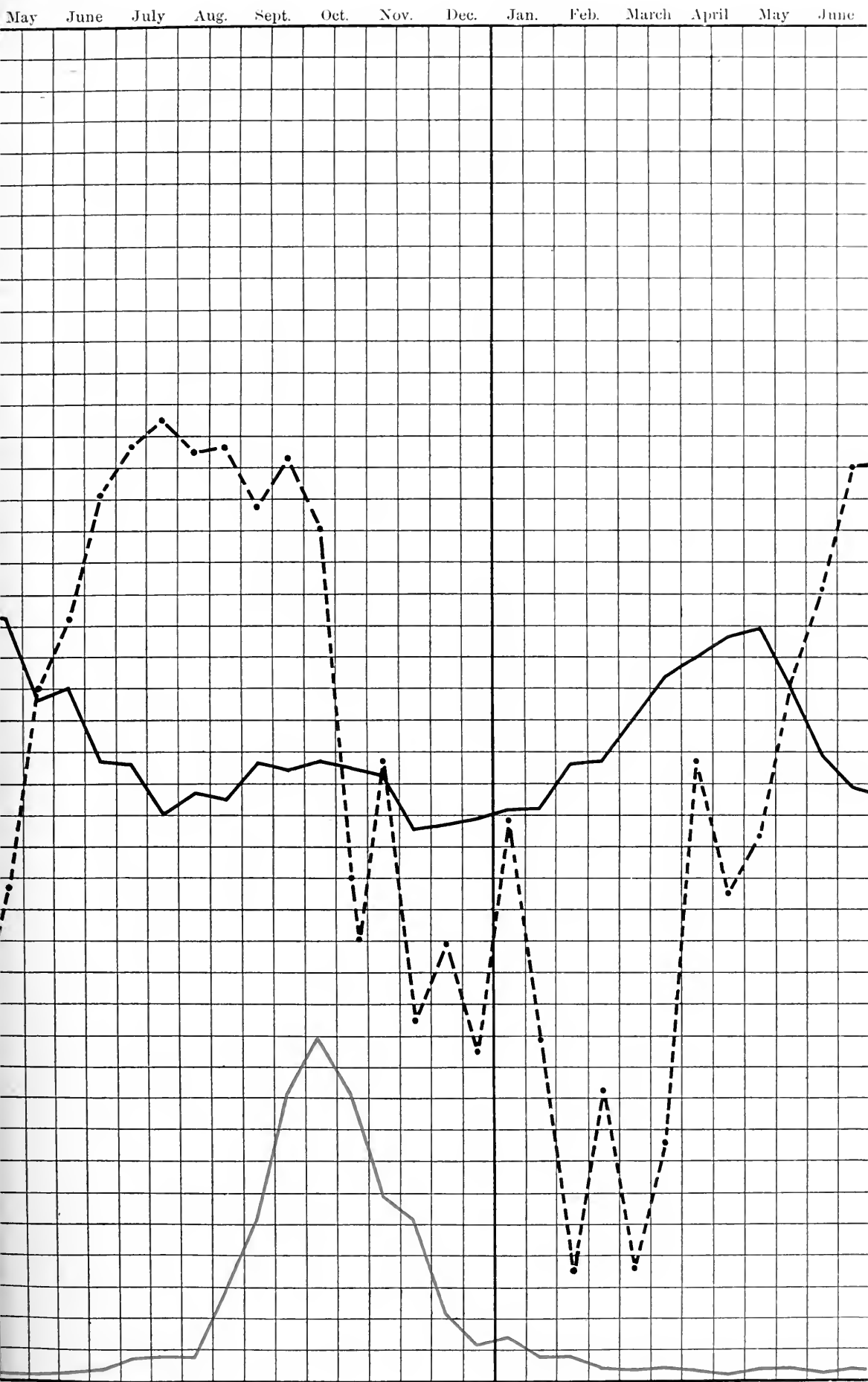


CHART IV (continued)

1904



BELGAUM, 1897—1906

- Plague deaths
- Mean temperature
- Humidity

27
35
CHART IV (continued)

BELGAUM,

1897—1906

1905

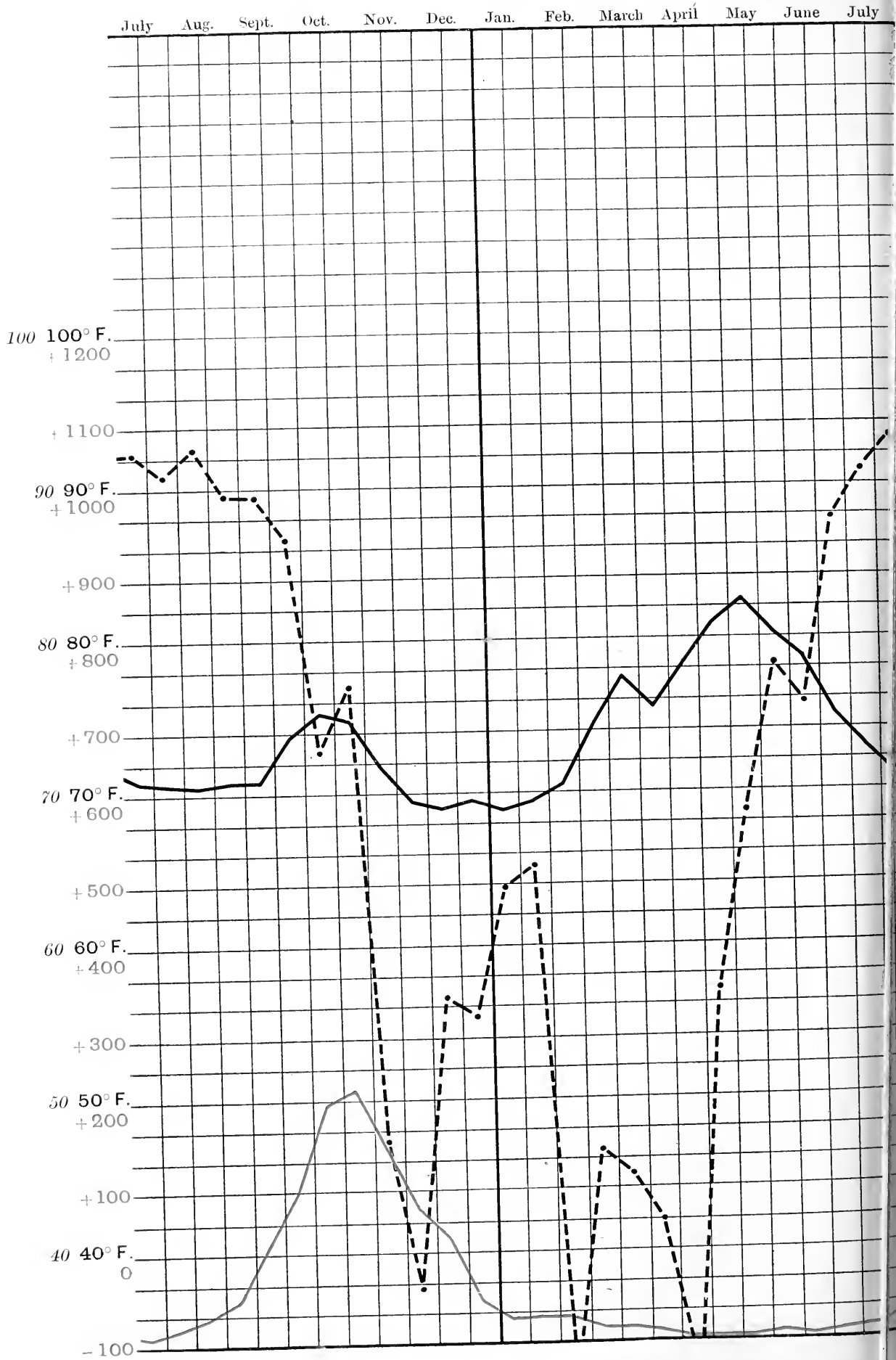
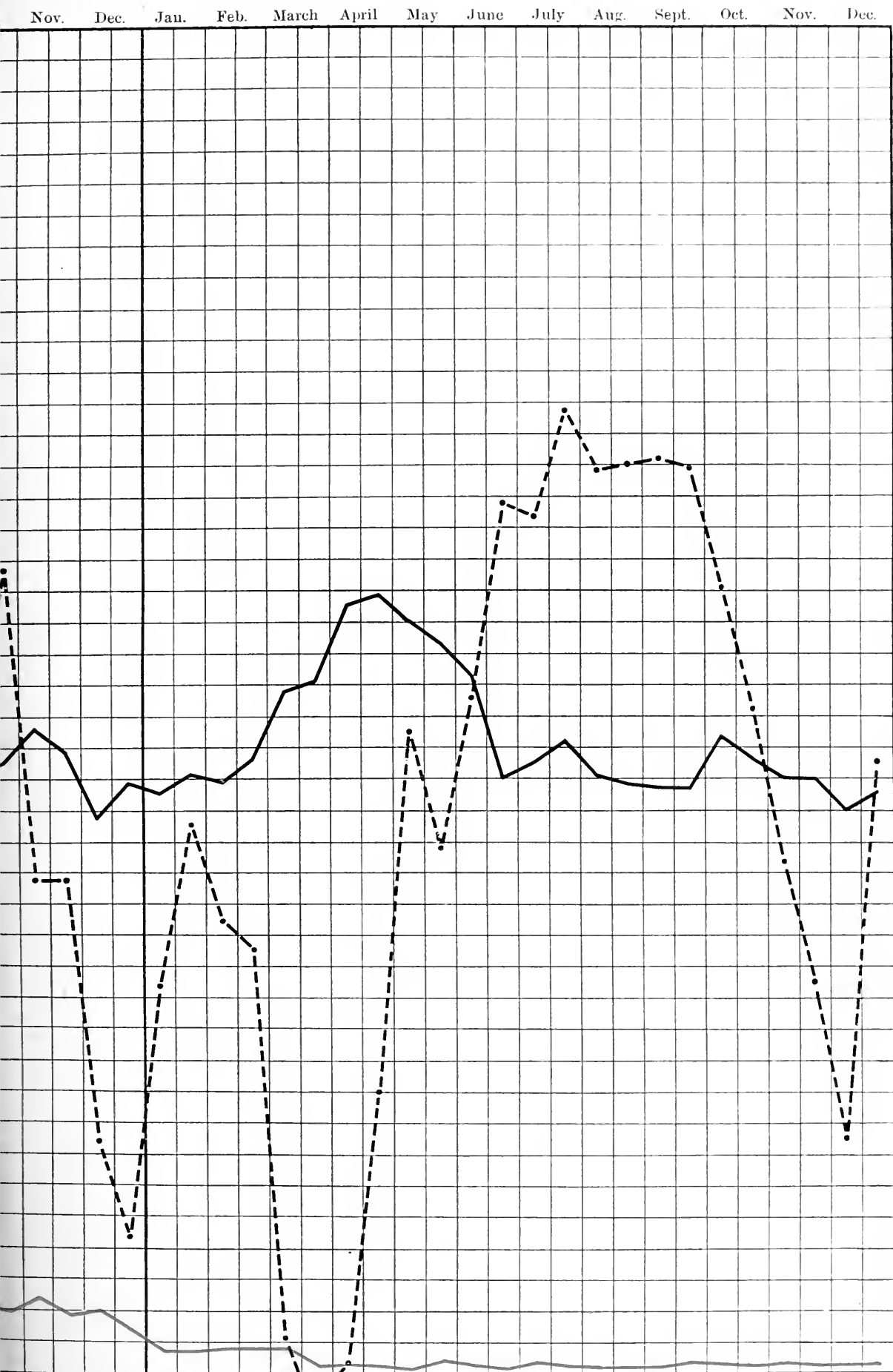


CHART IV (continued)

1906



BELGAUM, 1897—1906

- Plague deaths
- Mean temperature
- Humidity

CHART V

LAHORE, PUNJAUB,

1902—1906

1902

1903

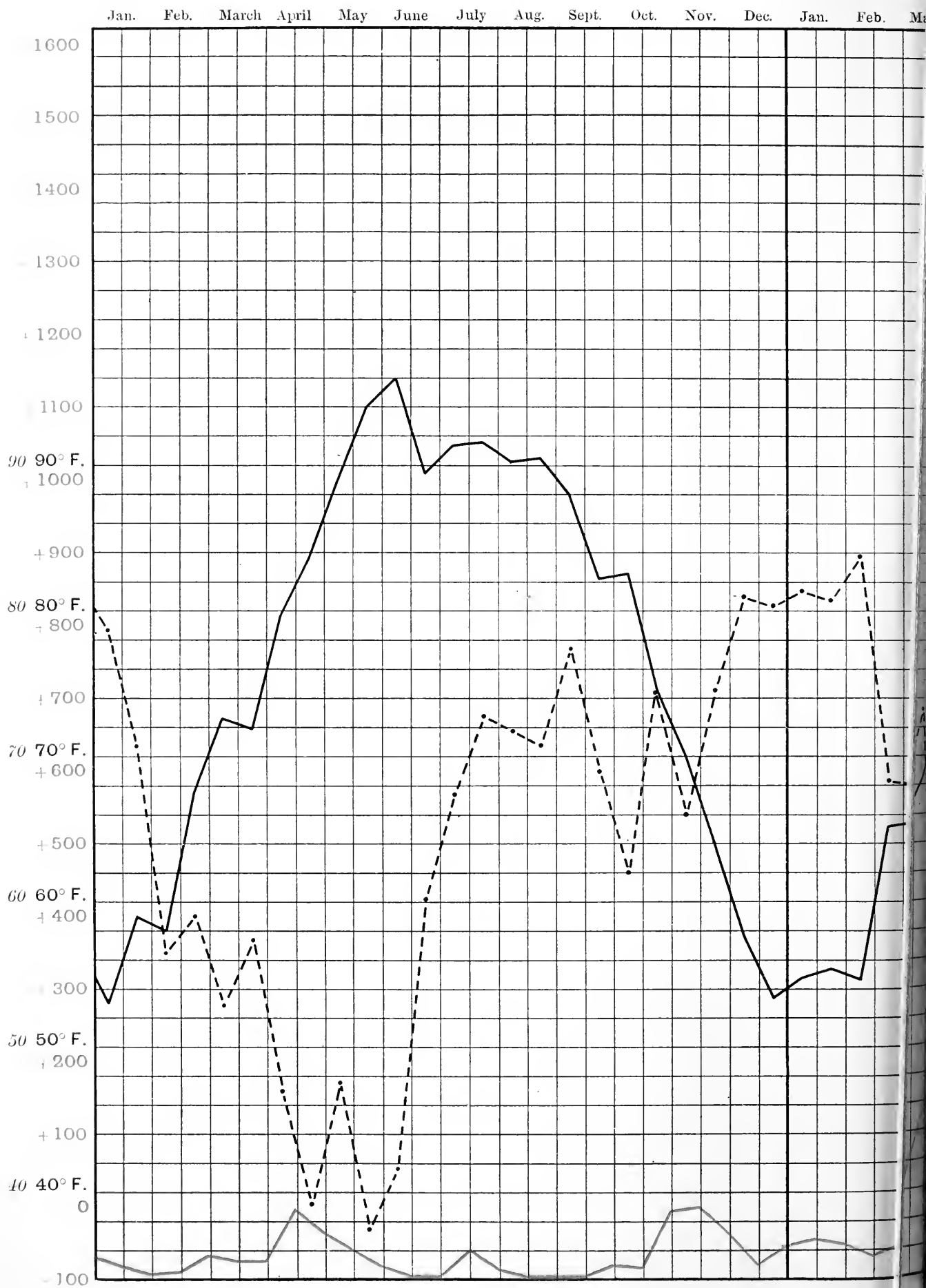
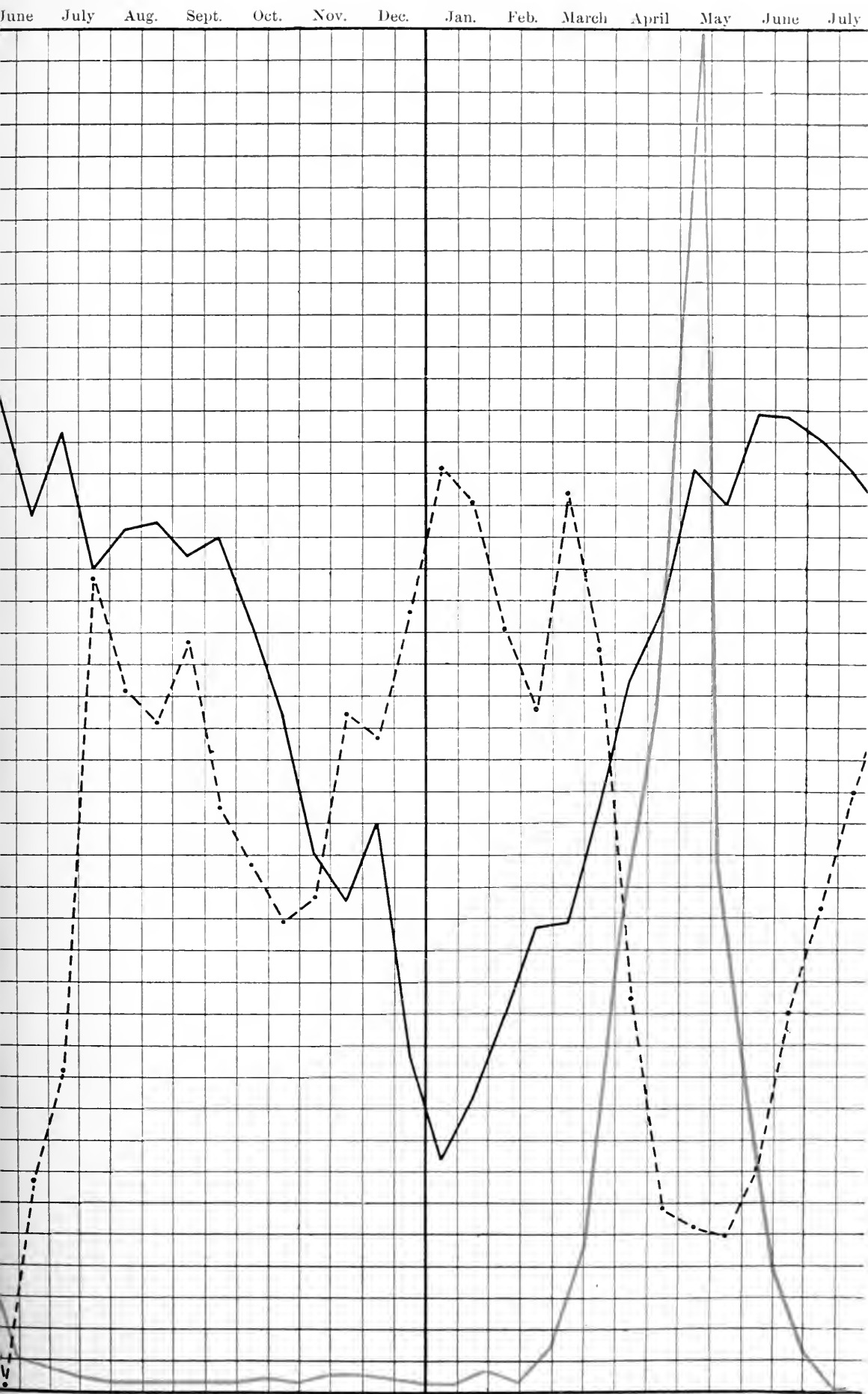


CHART V

1904



LAHORE, PUNJAUB, 1902—1906

- Plague deaths
- Mean temperature
- Humidity

CHART V (continued)

LAHORE, PUNJAUB,

1902—1906

1905

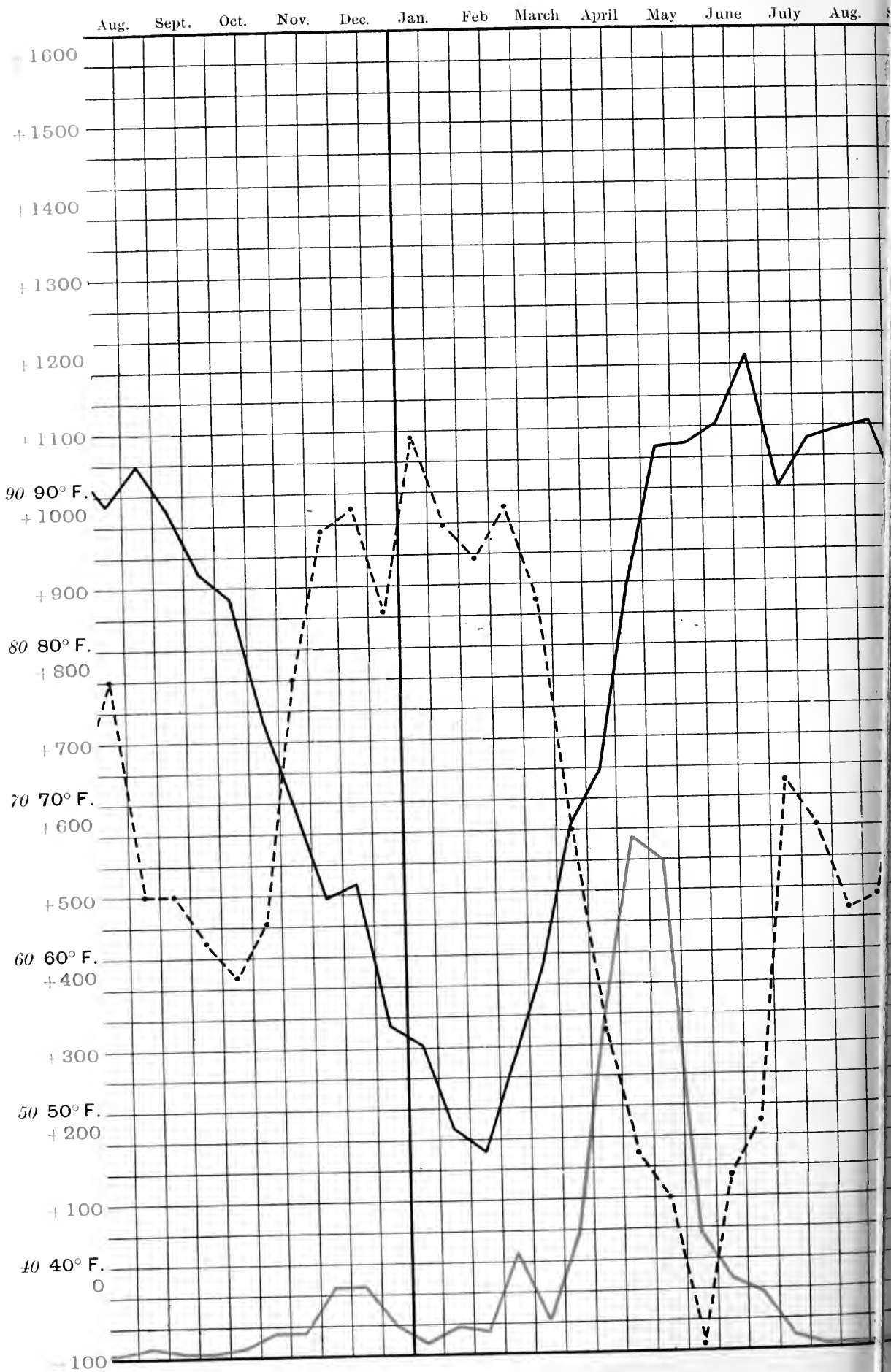
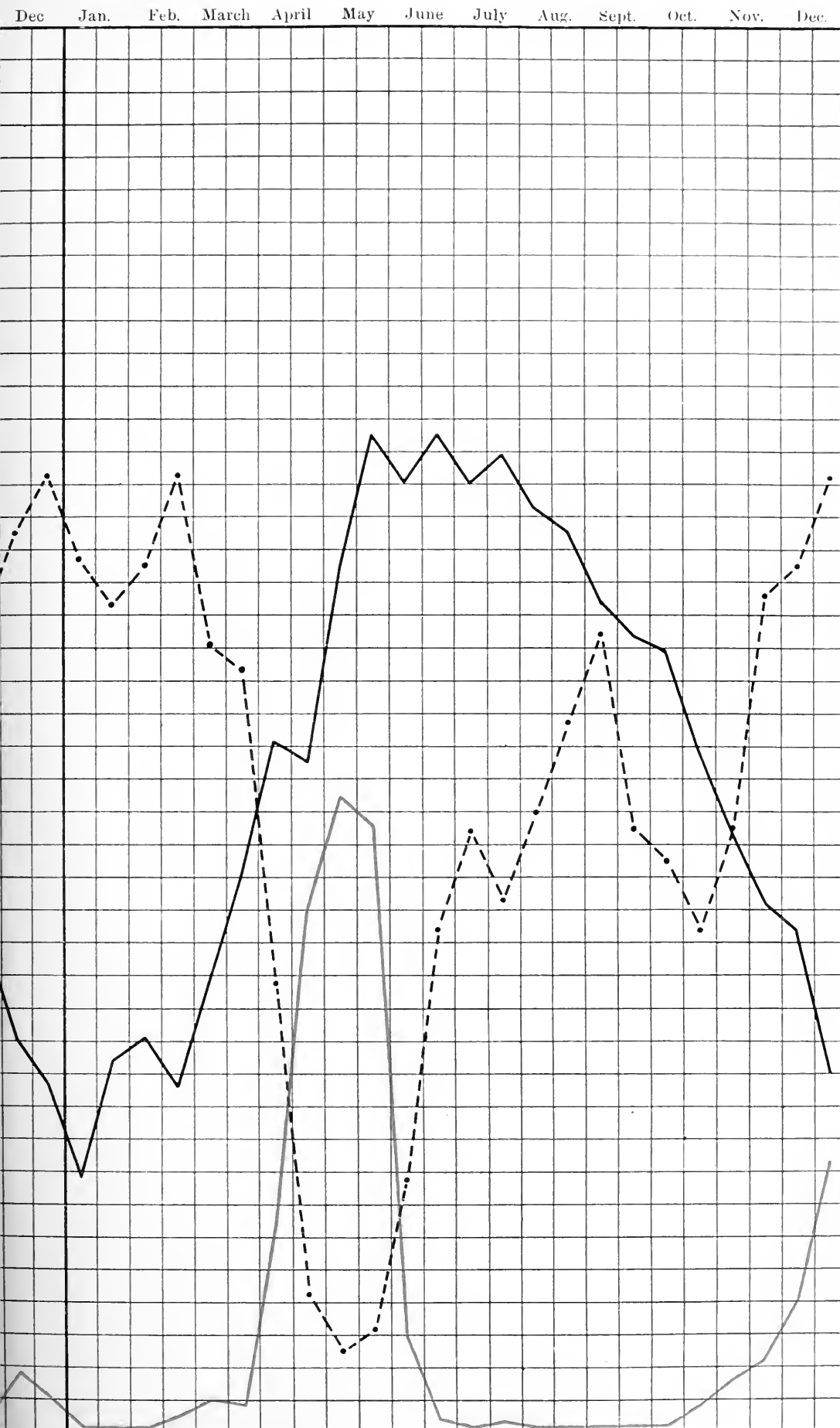


CHART V (continued)

1906



LAHORE, PUNJAUB, 1902—1906

- Plague deaths
- Mean temperature
- - - - Humidity

CHART VI

RAWALPINDI, PUNJAUB,

1903—1906

1903

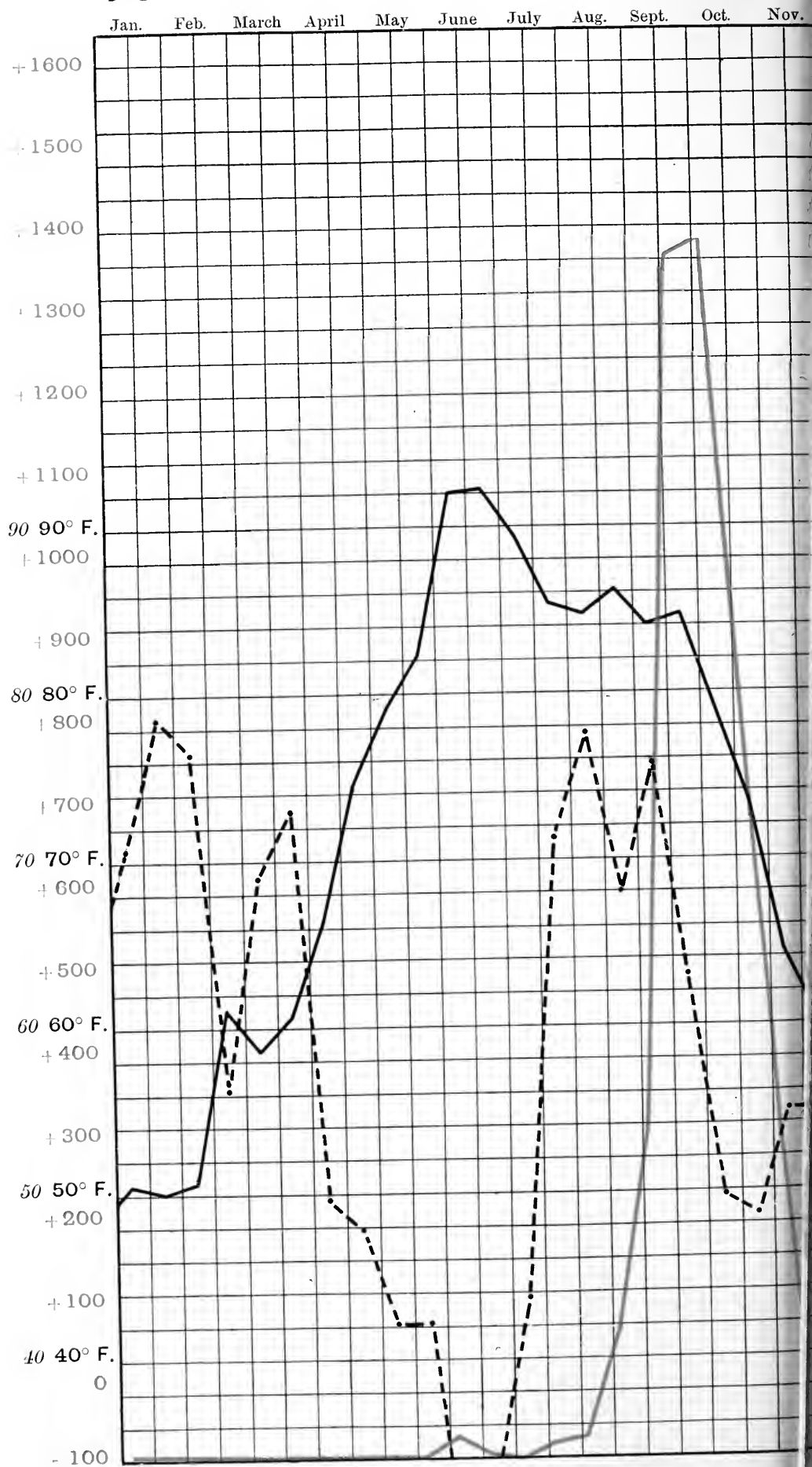
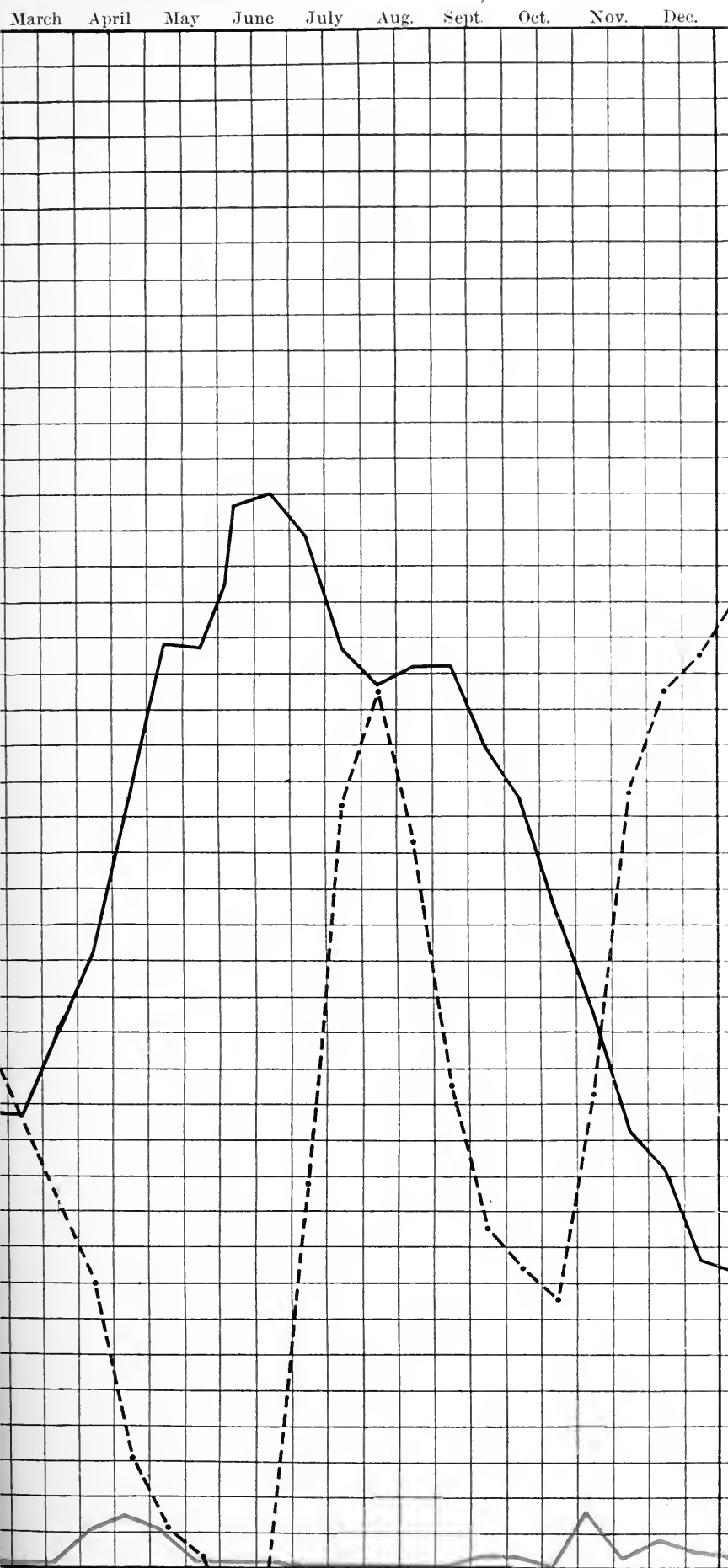


CHART VI



RAWALPINDI, PUNJAUB, 1903—1906

- Plague deaths
- Mean temperature
- Humidity

CHART VI (continued)

RAWALPINDI, PUNJAUB,

1903—1906

1905

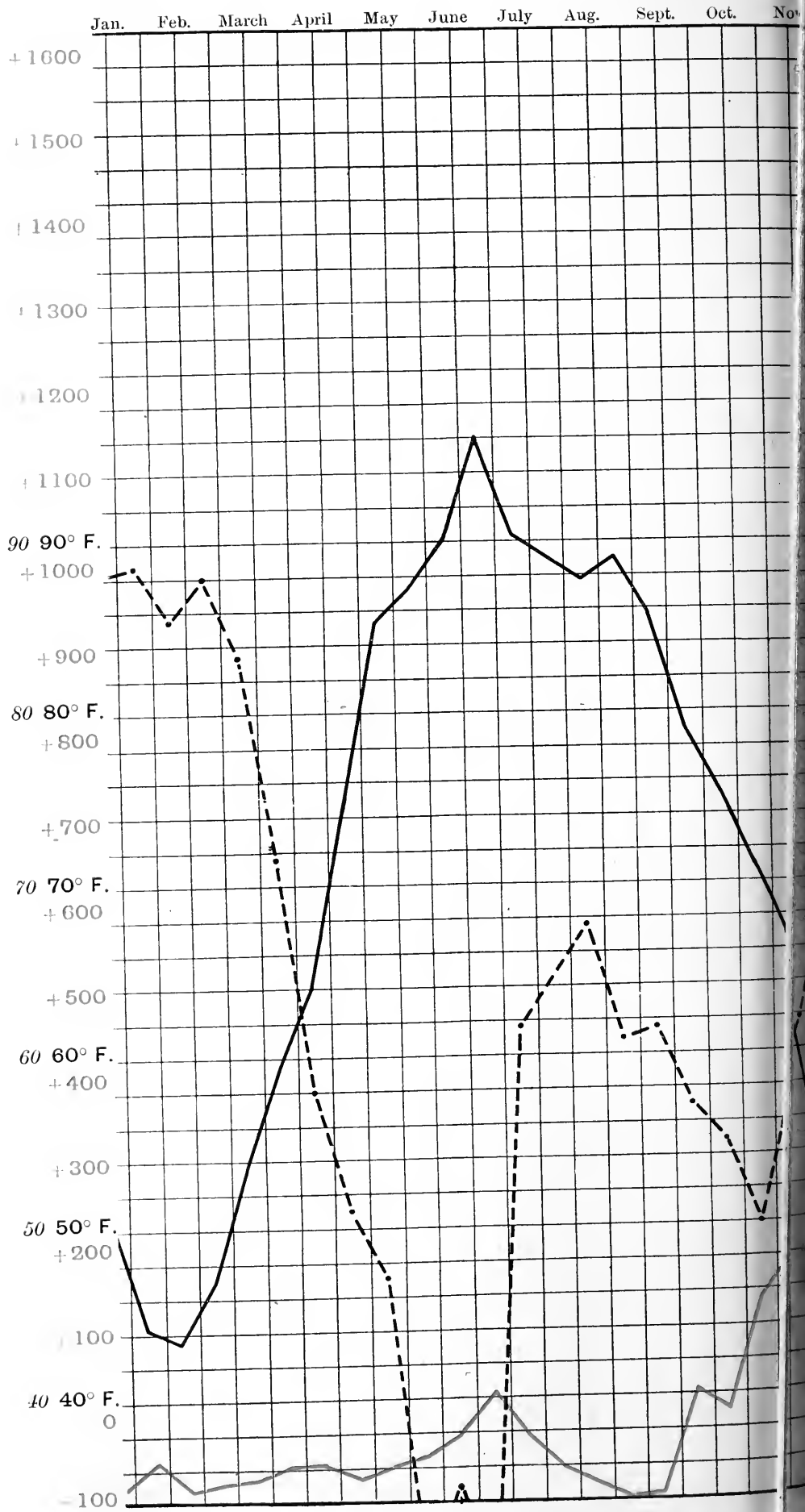
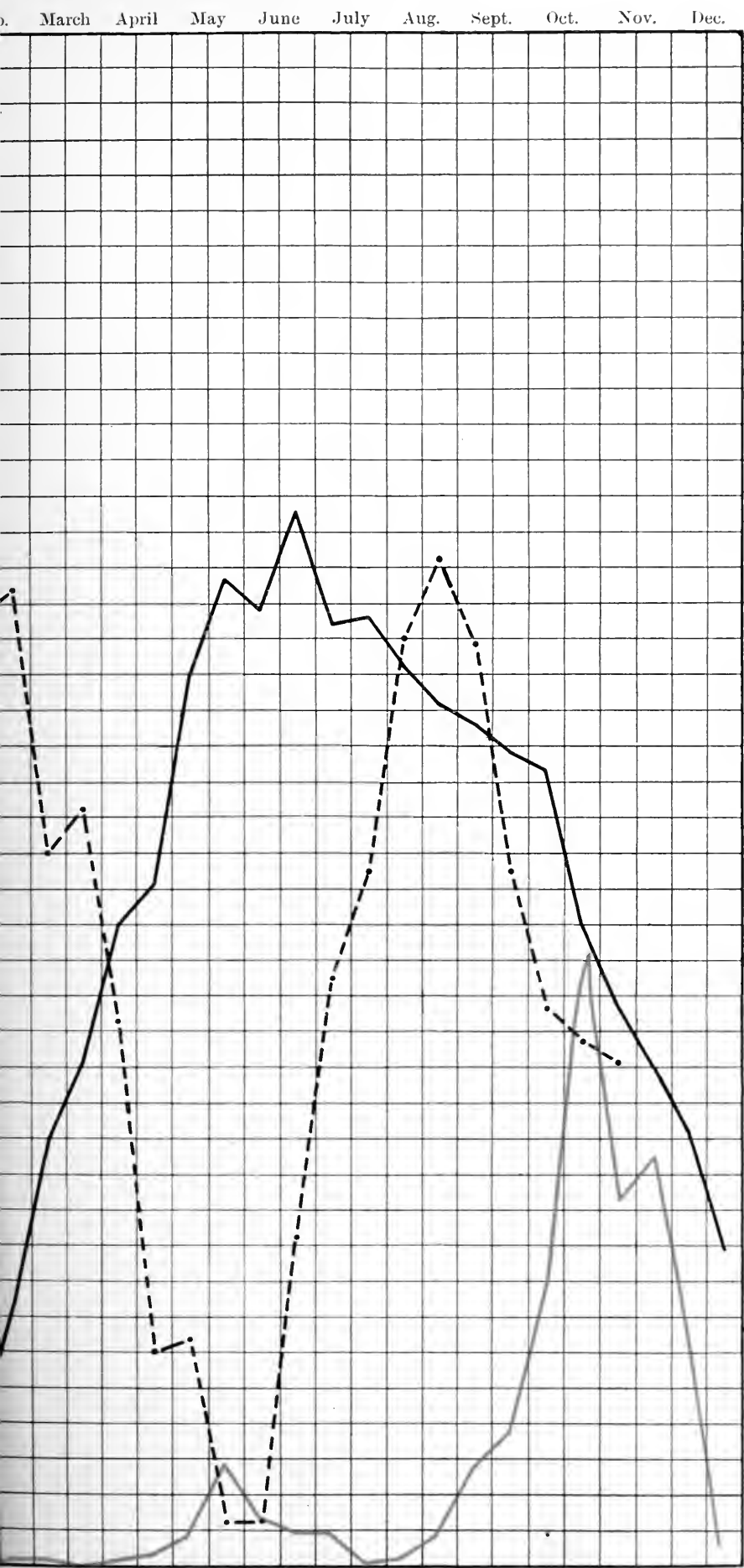


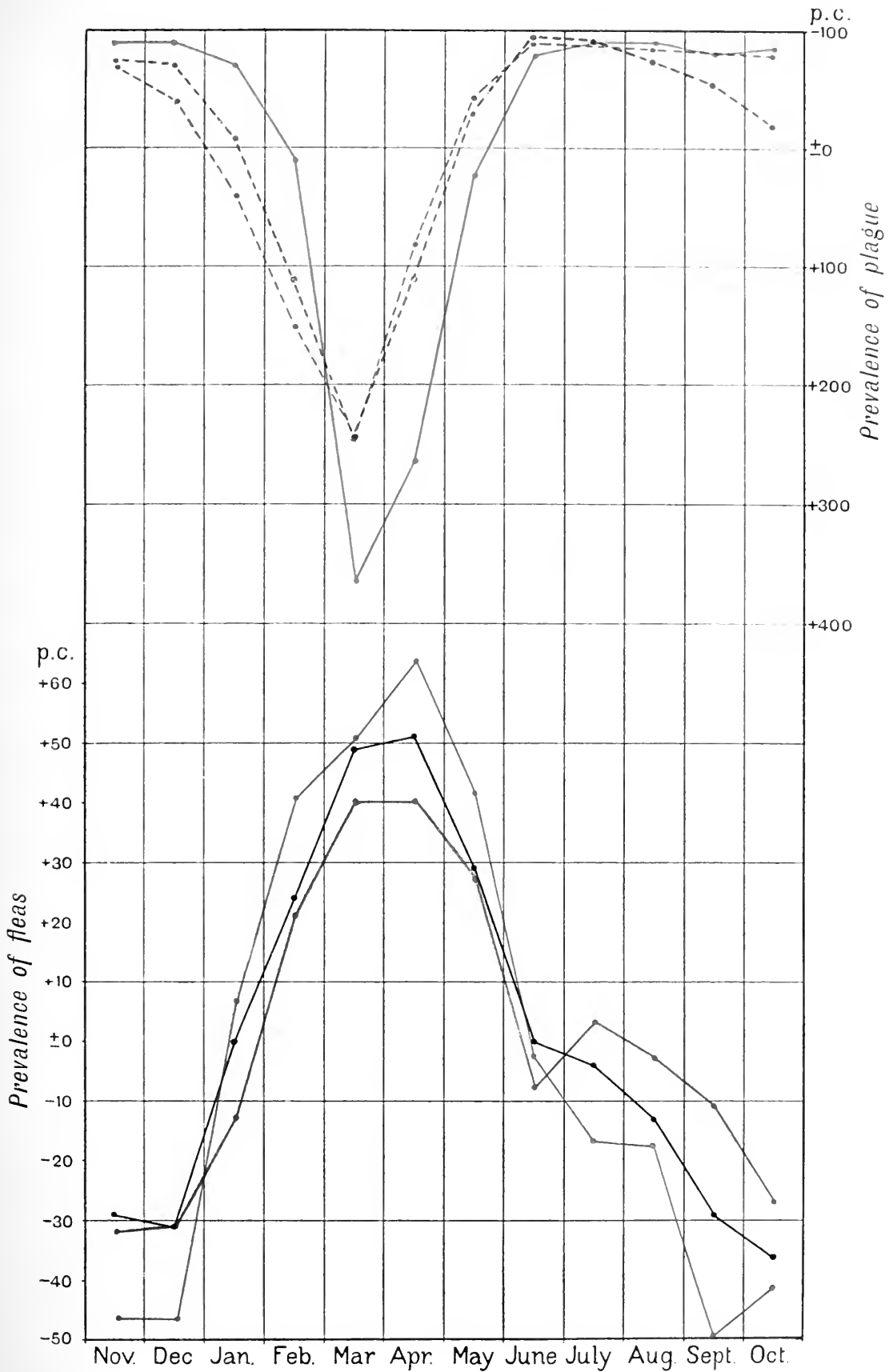
CHART VI (continued)



RAWALPINDI, PUNJAUB, 1903—1906

- Plague deaths
- - - Mean temperature
- Humidity

CHART VII



PREVALENCE OF FLEAS IN BOMBAY

- | | |
|-------------------------------------|--------------------------------|
| — Human plague | — Fleas on all rats |
| - - - Plague in <i>M. decumanus</i> | — Fleas on <i>M. decumanus</i> |
| Plague in <i>M. rattus</i> | — Fleas on <i>M. rattus</i> |

BACILLUS DYSENTERIAE OF FLEXNER IN RELATION TO ASYLUM DYSENTERY.

BY H. T. S. AVELINE, A. E. BOYCOTT AND W. F. MACDONALD.

(From the Lister Institute of Preventive Medicine.)

○ IN this country observers are familiar with dysentery as occurring on the one hand in asylums, where it is common [see Mott (1905), Knobel (1906), Candler (1907)], and on the other as scattered sporadic cases (often known as "ulcerative colitis") among the general population with an occasional small epidemic. The bacteriology of these conditions does not appear to have yet been placed upon an altogether satisfactory footing. Of the sporadic English cases little seems to be known beyond the fact that Foulerton (1902) found that the serum of several cases agglutinated Shiga's bacillus. In the case recorded by Saundby and Hewetson (1906) no evidence is adduced that the organism isolated was a dysentery bacillus at all. There is however clear evidence that the institutional disease is associated with *B. dysenteriae*. Hewlett (1904) obtained positive agglutination results with Flexner's bacillus in two cases which were negative with the Shiga type. Eyre (1904) in an important research examined nine acute cases from a London County asylum and isolated Shiga's bacillus from the faeces of 4 out of 5 examined during life, and from 2 out of 4 examined only post-mortem. He further showed that the patients' serum agglutinated Shiga's bacillus while it was without action upon the Flexner organism. Dr Eyre has been good enough to inform us that he met with Flexner's bacillus in 6 of the 9 acute and in 15 of 35 chronic cases. The chronic cases in no instance yielded Shiga's bacillus, and their serum did not agglutinate Shiga or Flexner, which latter organism at the time he regarded as of doubtful import. Finally, McWeeney (1906) isolated from a case of acute fatal asylum dysentery in Ireland an organism which appears to be the *B. dysenteriae* of Flexner

though it is stated to have produced no indol and to have been pathogenic for rabbits. The work of Morgan (1906, 1907) has shown that no sort of *B. dysenteriae* is responsible for the summer diarrhoea of children in London as is the case in America and elsewhere.

The present investigations have been carried out on material derived from patients in the Somerset and Bath Asylum at Cotford, near Taunton. The material was of necessity not quite fresh when received in London, but the results indicate that dysentery stools may be subjected to a satisfactory bacteriological examination when they have been passed for fully 24 hours.

In all cases surface plates of MacConkey's bile-salt-lactose-neutral-red agar were used for the separation of the organisms. It is more satisfactory to plate directly when the stool is mucous in character, or from a fresh emulsion when faecal, than after a preliminary incubation in a fluid medium. It was for example found in one of the early cases that plates made directly from the stool showed colonies of *B. dysenteriae* and of *B. coli* in the proportion of 100 to 16, while plates made from an 18 hours culture of the same stool in glucose-bile-salt peptone-water or in ordinary broth showed ten times as many *B. coli* as *B. dysenteriae*. From such plates the non-lactose-fermenting colonies were picked off and tested by cultural and agglutination methods. The colonies of *B. dysenteriae* on MacConkey's agar are to some extent characteristic in that each white colony is surrounded by a clear zone distinctly more yellow (i.e. more alkaline) in colour than the rest of the medium. Of the cultural tests most use was made of the fermentations of various sugars and alcohols, of the reaction in litmus-milk and of the production of indol. Practically, however, it is most convenient to make broth cultures of any suspicious colonies, examine for motility after four to six hours, and, if found non-motile, immediately apply the agglutination test. In this way a reasonably certain diagnosis may be arrived at in 24 hours, and afterwards confirmed by culture.

The serum used for agglutination in these cases was a horse serum prepared for therapeutic use with a number of strains of dysentery bacilli. These included the types of Shiga, Kruse and Flexner, those isolated by Eyre from asylum dysentery and several from cases of infantile diarrhoea in America. It agglutinated the homologous organisms of the Shiga and Flexner types up to a dilution of 1:10,000 and was generally used diluted 1:1000, the observations being made microscopically at room temperature; in all cases a positive result was reached in two hours if at all.

Clinically the cases examined on the whole conformed to the usual type of acute asylum dysentery. As a rule the onset was sudden with headache, pains in the back and limbs and malaise ending in vomiting: then a rigor or an abrupt rise of temperature to 102° — 104° F. Diarrhoea then begins: at first the stools appear normal, then loose and generally after 24 hours small containing blood and slime. Epigastric pain is complained of during the first few days, later pains in the region of the large intestine aggravated by palpation. The stools have a characteristic sour odour and contain greyish-white, tenacious slime intimately mixed with blood: six or eight motions in 24 hours is about the average for the first few days. The average duration under treatment was about 8 days, the temperature reaching normal about the third day, after which the number of stools diminishes. Slime persists in the stools after blood has ceased to be passed.

Working in this way, material from 44 inmates of the asylum was examined: 20 of them had dysentery at the time. The results may be grouped as follows:

(a) Of the cases of clinical dysentery, the stools were examined in 19 and *B. dysenteriae* of the Flexner type was in all isolated from 17. In one fatal case the spleen and mesenteric glands were alone available: *B. dysenteriae* was found in both. Three of the positive cases died: in one *B. dysenteriae* was isolated from the stools 14 days before, and also on the day of death, but was not found in the ulcerated coecum post-mortem; in the second it was found in the stools four days before death and also recovered from scrapings of the mucosa of the coecum; in the third death occurred $2\frac{1}{2}$ months after dysentery bacilli had been found in the stools and they were not recovered from the spleen. The stools of five of the positive cases were examined during convalescence: one was negative 13 and 28 days after the attack, one negative after 8 and 30 days, one negative 14 days after, and two negative 25 days later. One case was examined as a normal control two days before the onset of symptoms with a negative result; on the third day of illness *B. dysenteriae* was found in large numbers. Two others had also been previously examined with negative results two and three months respectively before the onset of acute illness. In three acute cases the stools failed to yield dysentery bacilli: in one however these were found 28 days later during convalescence but not 41 days after the acute attack. In another case the symptoms of dysentery continued for $4\frac{1}{2}$ months; the stools were examined three times and the spleen and mesenteric glands post-mortem—all with negative results.

(b) The stools were examined in 26 cases which were not clinically dysentery though five of them had diarrhoea. Twelve had had previous attacks of dysentery, in two 5 weeks before examination, in four

2 months, in two 3 months and in three 12 to 14 months. In one case scrapings from small ulcers found in the upper third of the rectum of a patient dying of nervous disease, who had had diarrhoea for three months before death, were examined. In none of these 27 cases was *B. dysenteriae* found. Most of them were contacts in the same wards with cases of dysentery which had been verified bacteriologically. There were for instance at one time three cases of dysentery so verified in one ward; while these persons were ill, stools from ten other patients in the same ward were examined: the results were all negative though three of them subsequently developed clinical dysentery and *B. dysenteriae* was found in their faeces.

The organisms isolated conformed in all respects to the *B. dysenteriae* of the Flexner type¹. They fermented dextrose, laevulose, galactose and arabinose with the production of acid without gas, did not change the reaction in lactose, dulcitol, erythritol, salicin or inulin, caused an initial acidity with a terminal alkalinity in litmus-milk, produced a slight to a moderate amount of indole (paradimethylamidobenzaldehyde test), were not motile and agglutinated with the multivalent anti-dysentery serum up to 1:1000 and, when so examined, up to 1:10,000. In no instance were any bacilli of the Shiga type found, and a few agglutination tests with the blood of the patients showed no reaction with Shiga's original strain or with Eyre's cultures, while with the bacilli isolated from the corresponding and other cases and with Flexner's strain a good reaction up to a dilution of 1:200 or 1:500 was sometimes obtained.

In the differentiation of dysentery bacilli further than into the broad divisions of Shiga type (not fermenting mannite) and Flexner type (fermenting mannite), some importance has been attached by Hiss and others to the reactions in media containing maltose and cane-sugar. These authors note that to some extent the reactions are inconstant, especially as to the time at which the acid reaction may appear. The following tests were made simultaneously on 24 cultures from seven cases of the present series. The test sugars were in 1% solution in a mixture of one part peptone beef broth with three parts peptone-water; incubation was at 36° C. The number of cultures which were acid at different times was as follows:

Days incubated	Cane-sugar				Maltose			
	1	7	14	28	1	7	14	28
Number of cultures acid	0	0	1	4	2	3	13	24

¹ See the papers of Hiss (1904), Torrey (1905), Shiga (1906) and Dopter (1907).

There can be little doubt that all these cultures must be regarded as being essentially the same bacillus. Even if that were not the case, it is difficult to believe that all the cultures from any one case are not the same. Yet the variations of fermentative power in the whole series of cultures do not in any way correspond to the distribution of the cultures among the cases. Thus the four cultures which eventually produced an acid reaction in the cane-sugar medium came from four different cases, and the three which fermented maltose in seven days also from three different cases.

SUMMARY.

1. *B. dysenteriae* of Flexner has been found in the stools of 17 out of 19 cases of asylum dysentery; no evidence of the presence of the Shiga type was obtained.

2. In 18 cases examined one week to 14 months after an attack of dysentery, *B. dysenteriae* was found only once (3 weeks).

3. No evidence has been obtained of the presence of *B. dysenteriae* in the faeces of ward contacts (26 cases) with either normal or diarrhoeic stools.

4. The fermentative reactions of *B. dysenteriae* of Flexner towards maltose and cane-sugar are variable.

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AGGLUTINATION OF BACILLI OF THE ALKALIGENES, COLON AND TYPHOID GROUPS BY THE BLOOD SERUM OF CASES OF CEREBRO-SPINAL FEVER.

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By means of a method introduced by one of us (Wilson, 1907), a bacillus closely resembling *B. faecalis alkaligenes* was recently isolated from Belfast tap water. This bacillus, for the purposes of this paper, will be spoken of as *B. Grosvenor*, it having been attained from a house in Grosvenor Road. We find that emulsions of this bacillus are agglutinated, often in high dilutions, by the blood serum from practically every case of the cerebro-spinal meningitis at present existing in epidemic form in Belfast.

That this epidemic is associated with Weichselbaum's *Meningococcus* we have pointed out in a former paper (22. vi. 1907).

Briefly the characters of the *B. Grosvenor* are as follows:—an actively motile bacillus; Gram-negative; gelatin not liquefied; no gas in glucose broth, nor with any other sugar or alcohol tested; milk not coagulated; very faint trace of indol in peptone water; neutral-red agar not fluorescent; Petruschky's litmus whey faintly alkalinised; Barsiekow's two media slightly alkalinised, then bleached, the blue returning on shaking; bluish colonies with faint reddish centre on Drigalski-Conradi plates; brown growth on potato, and abundant growth on agar.

A fairly highly agglutinating anti-typhoid serum clumped the bacillus in dilutions of 1 in 50, but in no higher dilution, whereas the same serum readily clumped *B. typhosus* in 1 in 1000 dilution in 15 minutes.

Our attention was strongly drawn to the *B. Grosvenor* by the following circumstances: six samples of blood were sent to us to be tested by the Grünbaum-Widal test for typhoid fever; these samples were found to be negative as regards agglutination of *B. typhosus* but one of them clumped *B. Grosvenor* in a dilution of 1 in 1000 and, on enquiry, this blood was found to have been taken from a case of epidemic cerebro-spinal meningitis, from the spinal fluid of which we isolated the specific *Meningococcus*. Moreover, this blood did not agglutinate *B. coli* in a dilution of 1 in 50.

Led by this hint, we tested the agglutinating power of normal human blood serum on *B. Grosvenor*, taking the blood from 31 healthy adults, with the result that three of the number, i.e. 9·6 %, gave a positive reaction in dilutions of 1 in 50, but none gave clumping in 1 in 100. As a further control we tested the blood of 83 patients (non typhoid) from the wards of a general hospital, including such conditions as bronchitis, nephritis, tuberculosis, neuritis, acne, chronic rheumatism, dyspepsia, myocarditis and measles, and found that 56 were negative to 1 in 50, 25 were positive at this dilution, but negative to 1 in 100, while two only were positive to 1 in 100, and of these two one suffered from bronchitis, the other from furunculosis. As a still further control blood serum from 41 cases of enteric fever was tested and it was found that 24 were negative to *B. Grosvenor* in dilutions of 1 in 50, 10 were positive at this dilution, one was positive to 1 in 100 dilution; in the remaining six the reaction was doubtful.

Thus out of the above 155 cases used as controls there were three cases only in which the serum agglutinated *B. Grosvenor* in a dilution of 1 in 100; and 44 which gave a positive result in a 1 in 50 dilution; none of the controls clumped the bacillus in dilutions above 1 in 100.

In marked contrast to this result was the effect of serum from cases of cerebro-spinal fever upon *B. Grosvenor*, thus: blood serum from 135 of these cases acted as follows:—121 gave clumping (many of them instantaneously) in 1 in 50, 10 gave no clumping when first examined but when a fresh sample was tried on a subsequent occasion, the clumping occurred in some cases even in dilutions of 1 in 1000; lastly the remaining four were negative and the death of these patients prevented a subsequent re-testing.

As mentioned, many of the cases gave instantaneous agglutination in dilutions of 1 in 50, so that we were encouraged to push the dilutions to a higher degree, and found that 12 of these cases gave marked clumping within an hour to dilutions of 1 in 1000, and further, we

found four of the twelve agglutinated in dilutions of 1 in 1600, and four others in dilutions of 1 in 1200, 1 in 1400, and 1 in 2000 respectively.

These higher dilutions were practised with blood taken a second and third time and oftener at intervals from the same patient, it being apparent that the agglutinin increase more and more as the patient progresses in convalescence, or rather in chronicity, seeing that death has ultimately occurred in some of these cases.

In the next place the agglutinative effect of blood serum from undoubted cases of cerebro-spinal meningitis was proven upon a flaginac colon bacillus which had been isolated from the urine of a cerebro-spinal case. Eighteen different samples of blood were so tested and it was found that 16 of them gave no clumping in dilutions of 1 in 50 while one of these bloods, in dilutions of 1 in 1000, clumped *B. Grosvenor*. One sample gave feeble clumping at 1 in 50. The remaining sample clumped the *B. coli* at 1 in 400, but although agglutinating *B. Grosvenor* at 1 in 50 was quite inactive at 1 in 400; it also clumped *B. typhosus* at 1 in 50 dilution, but not at 1 in 100.

Similarly the serum from 21 cases of cerebro-spinal fever was tried with *B. typhosus*. Fourteen of these did not clump Eberth's bacillus in dilutions of 1 in 50, although several of them agglutinated *B. Grosvenor* when diluted 1000 times. The other seven samples gave excellent clumping with *B. typhosus* as shown in Table II.

TABLE II. *Shows agglutinating effect of blood serum of cerebro-spinal fever cases on B. typhosus.*

(The sign + indicates a positive reaction, + + marked agglutination, - indicates no clumping.)

Name of patient	Dilutions				Remarks
	1 in 50	1 in 100	1 in 200	1 in 400	
1. L—s	+ +	-			<i>B. Grosvenor</i> 1:50 positive, 1 in 200 negative. <i>B. coli</i> 1 in 50, 100, 200, and 400 positive.
2. McA—er	+ +	-			<i>B. Grosvenor</i> 1 in 200 positive. <i>B. coli</i> 1 in 50 negative.
3. G—ble	+ +	+ +	+	-	<i>B. Grosvenor</i> 1 in 200 positive.
4. McG—n	+ +	+ +	+ +	+ +	<i>B. coli</i> 1 in 50 negative.
5. McG—re	+ +	+ +	+ +	-	<i>B. Grosvenor</i> 1 in 200 positive. <i>B. coli</i> 1 in 50 negative.
6. W—son	+ +	-			
7. Cl—son	+ +	+ +	+ +	-	<i>B. Grosvenor</i> 1 in 200 positive. <i>B. coli</i> 1 in 50 negative.

The last case (number 7) in the above table was particularly interesting. The patient was a strongly built woman, 27 years of age.

Clinically her symptoms were typically those of meningitis, and yet her blood on three different occasions, when tested against *B. typhosus*, gave a marked agglutinative effect in dilutions of 1 in 200, both the microscopic and macroscopic methods being employed, as well as two separate strains of *B. typhosus*. An autopsy was permitted by her father, who informed us that she had never had any illness since an attack of scarlet fever in her ninth year. The post-mortem showed an advanced purulent inflammation of the meninges of the brain and spinal cord, from the pus of which the *Meningococcus* was recovered in pure culture. Moreover, the thoracic and abdominal organs presented the appearances so often found in cases of epidemic cerebro-spinal fever (see Symmers, Radmann, Westenhoefer) particularly the gastro-enteritis with enlarged solitary follicles and swollen and hyperaemic mesenteric glands. Peyer's patches were normal, the spleen was about a third larger than normal. The spleen, mesenteric glands and urine were exhaustively examined by cultural methods for *B. typhosus*, but none were obtained. This case is in various particulars similar to one obtained by Cowie (1907) who, relying, very naturally, on the positive Widal reaction, regarded his case as one of enteric fever with meningeal symptoms. Dr Cowie's report of the post-mortem lesions in his case are also very interesting in their similarity to those mentioned above.

In addition to the cases of cerebro-spinal fever occurring in Belfast we have been able, through the kindness of Dr Darling, of Lurgan, Dr Johnston, of Glasgow and Dr Ker, of Edinburgh, to examine blood coming from these three centres respectively. A glance at Table III will show that the results, judging from the few cases available, are along the same lines as mentioned in regard to the Belfast cases.

We thus see that there exist in some normal bloods substances that agglutinate the *B. Grosvenor*, that these substances are increased in some individuals suffering from general diseases, that in many cases of typhoid fever they undergo a further increase, but that it is in cerebro-spinal fever that the increase is constant, striking and progressive. We may remark that *B. Grosvenor* on agar furnishes a perfect emulsion that shows no tendency to spontaneous clumping, but that a specimen of *B. faecalis alkaligenes* (kindly sent to us by Dr A. C. Houston) was, owing to spontaneous clump formation, unsuitable for agglutinative work.

As far as our experiments have gone the blood of rabbits immunised with the *Meningococcus* does not agglutinate the *B. Grosvenor*. In view of the fact that we have never succeeded in isolating *B. Grosvenor* from

the bodies of cerebro-spinal fever cases, although a number of such attempts has been made, we content ourselves with merely stating the facts and refrain from all attempts at explanation.

TABLE III. *Shows the agglutinating effect of blood serum from cerebro-spinal cases from Lurgan, Glasgow and Edinburgh on B. Grosvenor.*

(+ indicates a positive reaction, ++ marked agglutination, - indicates no clumping.)

Name of patient	Date	Dilutions							Remarks
		1 in 50	1 in 100	1 in 200	1 in 300	1 in 400	1 in 600	1 in 800	

<i>Lurgan cases.</i>									
1. Fr—s	20/3/07	+	+	+	+				} From the spinal fluid of all these cases the <i>Diplococcus intracellularis meningitidis</i> was isolated.
2. B. G.	20/3/07	+	+	+	+				
3. McJ—y	19/5/07	+	+	—					
	23/5/07	+	+	+	+				

<i>Glasgow cases.</i>									
1. P—er	15/5/07	+	+	+	+	—	—	—	} From the spinal fluid of all these cases the <i>Diplococcus intracellularis meningitidis</i> was isolated.
2. W—r	15/5/07	+	+	+	+	+	—	—	
3. F—lay	15/5/07	+	+	+	+	+	+	+	
4. L—y	15/5/07	+	+	+	+	—			
5. K—y	15/5/07	—	—						

<i>Edinburgh cases.</i>									
1. A case of posterior basic meningitis	19/5/07	+	+	—					
2. Ed. I. c. s. f.	23/5/07	+	+	+					
3. Ed. II. c. s. f.	23/5/07	+	+	+					

Having thus determined that the serum of cerebro-spinal cases has an agglutinative effect on *B. Grosvenor* and that the serum of rabbits infected with the *Meningococcus* has no effect on this bacillus we proceeded to show that the Grosvenor-agglutinins in the blood of patients suffering from cerebro-spinal meningitis are separate and distinct from the meningococcus-agglutinins present in the patients. This distinction between these two agglutinins is evident from the following experiment:—from a certain patient affected with cerebro-spinal meningitis, a serum was obtained that agglutinated *B. Grosvenor* in dilutions up to 1 in 2000. One drop of this serum was added to 19 drops of 0·8 % saline solution, to this was added a loopful of an agar culture of *B. Grosvenor*, the mixture was allowed to stand at room temperature for two hours (or incubate at 37° C.), was then centrifuged and the supernatant fluid was found to be completely devoid

of agglutinating effect on *B. Grosvenor*. Whereas, the serum similarly diluted and treated, but with the addition of the *Meningococcus* in place of *B. Grosvenor*, retained its full agglutinative effect on *B. Grosvenor*. It is thus evident that saturation with meningococci has no effect on the agglutinin that acts on *B. Grosvenor*. Similarly it was found that saturation with *B. typhosus*, *B. coli*, or *B. faecalis alkaligenes* did not remove the agglutinin that acts on *B. Grosvenor*.

Conversely, we found that saturation with *B. Grosvenor* did not in any way alter the agglutinative effect of the serum as regards the *Meningococcus*.

Further Dr Houston determined for us the agglutinative and opsonic power of this serum after saturation respectively with (1) *Meningococci* and (2) *B. Grosvenor*;—the result being that saturation with *B. Grosvenor* did not diminish the opsonic power of the serum as regards *Meningococci*, neither did it lessen the degree of agglutination. Whereas saturation with *Meningococci* abolished the opsonic and agglutinative effect of the serum for *Meningococci*. This result is apparent in the following table:—

	Opsonic effect on <i>Meningococci</i>	Agglutination of <i>Meningococci</i>
Untreated serum	383 cocci in 40 leucocytes	++
Serum saturated with <i>B. Grosvenor</i>	401 „ „ „	++
„ „ „ <i>Meningococci</i>	27 „ „ „	—

Note: ++ means agglutination; the sign — means no agglutination.

In the next place we found that as a general rule the serum of cerebro-spinal cases when possessed of a high opsonic index as to the *Meningococcus* also showed high agglutinative power towards *B. Grosvenor*,—this relation of the two properties was however not invariable.

That the mere fact of suppuration did not explain the presence of agglutinin active toward *B. Grosvenor* was evident from the complete absence of such agglutination in three cases of suppurative meningitis that were respectively associated with *B. typhosus*, *B. enteritidis* Gaertner, and a pure streptococcal infection.

CONCLUSION.

1. The blood of patients suffering from epidemic cerebro-spinal meningitis is in practically all cases agglutinative to *B. Grosvenor*.
2. If the patient lives long enough, the reaction can be obtained in dilutions of 1 in 2000.

3. Occasionally the blood is agglutinative to *B. typhosus* and *B. coli* in comparatively high dilutions.

4. The opsonin and agglutinin acting on *B. Grosvenor* are quite distinct from those acting on *Meningococcus*.

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BILE SALT MEDIA AND THEIR ADVANTAGES IN SOME BACTERIOLOGICAL EXAMINATIONS.

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THE addition of bile salts to media was first employed by Ph. Limbourg (1889), who added the sodium salt of cholalic acid to a mixture of peptone and pancreas extract. He inoculated this medium with dog-faeces and investigated certain of the chemical changes which took place. Leubuscher (1890) used pure bile as a medium for the growth of *B. anthracis*, *B. typhosus*, and other bacteria and found it a good culture medium. Corrado (1891) grew certain organisms in pure bile and came to the conclusion

I. that it behaves

- (a) indifferently towards *B. typhosus* and *B. pneumoniae*,
- (b) as a stimulant to the growth of *B. mallei*,
- (c) bactericidally towards *B. anthracis*, which dies after 48 hours in bile.

II. that only in the case of the anthrax bacillus was a weakening of virulence noted. After 18 hours sojourn in bile this bacillus became non-pathogenic to animals.

Fraenkel and Krause (1899) concluded that *B. typhosus* suffered at any rate no loss of virulence during a 24 hours exposure to the influence of bile. They cite Mosse and also Falck as having used bile media. Matzuschita (1902) used a decoction of liver in preparing an agar medium and found it the best solid medium for faeces bacteria. Conradi (1906) strongly advocated the use of pure bile as a help towards the isolation of *B. typhosus* from the blood in cases of typhoid fever. Meyerstein (1907) added the salts of bile to a nutrient medium and grew various organisms in it. His results led him to the conclusion that :

- (1) *B. pyocyaneus* would grow well in a simple solution of bile salt,
- (2) the same solution, with a small amount of nutrient substance added to it, formed a good medium for the growth of *B. coli*, and
- (3) even the addition of large amounts of nutrient substances did not prevent it having an inhibitory action on the growth of *Staphylococcus pyogenes aureus*.

My first experiments were made in 1897 with a potato juice medium containing commercial bile salts; and these led to the suggestion in 1900 of bile salt lactose agar as a medium for *B. typhosus* and *B. coli communis*. The details of further experiments in which pure bile salts and bile acids were used were published in 1901 and in the same year Hill and I proposed bile salt glucose broth as a simple test for faecal contamination. A lactose broth had been previously used in our work for the Royal Commission on Sewage Disposal and glucose was substituted for the lactose merely so as to include such organisms as *B. enteritidis* (Gaertner). Of course, as stated by me (1904) in *Public Health*, if it is only a question of the presence of *B. coli communis* and its allies lactose broth is better than glucose broth.

The composition of the agar was as follows:—

Bile Salt	0·5%
Peptone	2·0%
Lactose	1·0%
Agar	1·5%
Tap Water	100 c.c.

and of the broth:

Bile Salt	0·5%
Peptone	2·0%
Glucose	0·5%
Litmus solution	q.s.
Tap Water	100 c.c.

On the agar, those organisms which decomposed the lactose produced acid in their immediate neighbourhood, and thus precipitated the bile salts with the consequent formation of a haze in the medium. This haze round the colonies differentiated them from those of non-lactose fermenters round which the medium remained quite clear.

Soon however it was observed that not every lactose-fermenting organism produced a haze when growing *on the surface* of the medium.

The reason for this will be apparent when Theobald Smith's work is referred to later on. At this time Grünbaum and Hume (1902) published their paper in which they proposed a modification of bile salt lactose agar. Their modification consists in the addition of 4 c.c. of $\frac{n}{1}$ NaHO to every litre of the agar and in the use of neutral-red as an indicator of the presence of acidity. They also say "the addition of Crystal Violet (1 in 100,000) permits of a striking double stain of the colonies." I had before this used neutral-red in bile salt broth for the "fluorescence test" but had not thought of it as an indicator. It seemed that the addition of this dye would do away with the difficulty occasioned by the absence of a haze round the surface colonies of some lactose fermenting organisms. It certainly acts very well as many colonies are pink and without a haze, but one still comes across surface colonies which, though colourless on neutral-red bile salt lactose agar, turn out to be colonies of lactose fermenting bacteria. The use of Crystal Violet is quite unnecessary as bile salt media alone inhibit most of the organisms found in air and soil (cf. MacConkey, 1900, p. 56) when the incubation temperature is 37° C. and over. The addition of 4 c.c. of $\frac{n}{1}$ NaOH per litre is to my mind a great disadvantage. The medium should be as neutral as possible to neutral-red, as otherwise organisms which attack the sugars, etc., only slowly and feebly may not be able to manufacture acid quickly enough and in sufficient quantity to overcome the alkalinity. Theobald Smith's observations are pertinent to this question. He says (1890) that in the absence of sugar both *B. coli* and the Hog Cholera bacillus are alkali producers. If a small quantity of sugar is present then, if the bacillus can act upon it, there is a rapid acid production and a slow alkali production. If the amount of acid is not too great it is neutralised by the alkali and the medium becomes alkaline. If the amount of acid is too great to be neutralised it tends to inhibit the growth of the bacillus. A similar result is obtained in the case of *B. typhosus*. Again in 1895 he states that more than 0.5% of glucose is harmful (cf. MacConkey, 1900, p. 48). The amount of acid produced is the same aerobically and anaerobically. There is a coincident production of alkali which is bound up with the growth of the organism, and which in the case of facultative anaerobes only goes on actively in the presence of oxygen. The acidity depends on the decomposition of sugar, etc.; the alkalinity, in the presence of oxygen, upon the growth

of the organism. He states further that if too little sugar be present the alkali production masks the acid production unless oxygen be removed.

My own observations entirely confirm these statements. An inoculated tube very rarely remains neutral; it almost always becomes either acid or alkaline. It may become acid and remain so; the initial acidity may be followed by alkalinity; or the appearance of alkalinity may be the first perceptible change. I have seen a red surface colony of *B. coli communis* on a litmus lactose agar plate gradually lose its redness and in the course of time become distinctly blue.

It is obvious therefore that the medium should be as neutral as possible to the indicator used¹. I have used both litmus and neutral-red and have come to the conclusion that the latter is to be preferred. It gives a lighter coloured medium and much better differentiation. Grünbaum and Hume's suggestion has been adopted by me, but not in its entirety. The added alkali has been omitted and the neutral-red accepted to the extent of using half the quantity mentioned in their formula.

The media are now prepared as follows. First a stock solution is made consisting of:—

Sod. Taurochol. (commercial from ox bile)	0·5%
Peptone (Witte)	2·0%
Water (distilled or tap)	100·0 c.c.

It is absolutely necessary to use bile salts which are neutral to neutral-red. Some samples are acid to this indicator and their use causes the finished medium to have a tint which makes the recognition of slight acidity somewhat troublesome. If litmus is preferred as an indicator it is not necessary to be so particular about the reaction of the bile salts. All the samples of bile salts I have tested have been alkaline to litmus. In this respect litmus has the advantage over neutral-red. Bile salts obtained from pig's bile are as far as my experience goes much more troublesome to use than those from ox bile. Calcium in the proportion of 0·03% of CaCl_2 seems beneficial to the growth of some of these organisms (cf. MacConkey, 1905, p. 336), and therefore this salt should be added when distilled water is used to make up the medium. Whenever possible I use tap water on this account.

¹ The use of test tubes made of glass which yields an appreciable amount of alkali to the contained liquid may cause the medium to become too alkaline.

When a solution of bile salts is mixed with a solution of commercial peptone a precipitate is formed (cf. Maly and Emich, 1883). There may be some difficulty in getting rid of this. The best way to obtain a clear solution is to mix the ingredients together, steam them for 1—2 hours in a Koch's steamer, filter while hot and then allow to stand (24—48 hours if possible) until quite cold and sedimentation has occurred. Filtration through paper will then remove the precipitate and yield a clear liquid, which appears to keep indefinitely under proper conditions.

The various bile salt broths are prepared from this stock solution by adding glucose 0·5%, lactose 1%, cane sugar 1%, dulcit 0·5%, adonit 0·5% or inulin 1%, and neutral-red (1% solution) 0·25%, distributing into Durham's fermentation tubes and sterilising in the steamer for 15 minutes on each of three successive days. I have never experienced any disappointment from using a bile salt medium which had been steamed only twice, but it is best to be on the safe side and steam three times. Great care must be taken not to heat above 100° C. or for too long a time after the addition of the sugars, as otherwise the medium may be spoiled. The amount of dulcit and of adonit has been decreased on account of the great increase in the price of these substances.

Bile salt agar is made by dissolving 2% of agar in the stock fluid either in the steamer or in the autoclave. The mixture is cleared with the white or the whole of an egg, filtered, neutral-red added in the same proportion as for broth, and distributed into flasks in quantities of 80 c.c., which is enough to make three plates of the usual size. When required for use the fermentable substance is added to the agar in the flask and the whole placed in a water bath or steamer. When melted the agar is poured into Petri dishes, allowed to solidify, and then dried in the incubator or warm room, the plate being placed upside down with the bottom detached and propped up on the edge of the cover. It is necessary that the surface of the agar should not be wet, as in that case the colonies will most likely form a confluent mass; nor should it be too dry as then the colonies are stunted in their growth. Inoculations are made by placing a loopful of a liquid culture or emulsion of the material to be examined on the centre of the agar in one plate and rubbing it over the surface with a bent glass rod; the same rod, without recharging, being used to inoculate the surface of the other two plates (Drigalski and Conradi's method). The plates are incubated upside down.

As regards the temperature of incubation 42° C. has of course a more selective action than one of 37° C., but the latter is quite good enough for ordinary purposes and is the temperature at which I have done all my work during the last four years. Therefore a special incubator is not necessary (cf. W. H. C. Forster, 1905).

Nor are anaerobic conditions of incubation absolutely essential. I have used them in the way of experiment to see if thereby any great advantage was to be gained, but I could not satisfy myself that the gain was sufficiently great to warrant the introduction of this method into routine work. From the observations of Theobald Smith, to which I have referred, it is obvious that the absence of oxygen may be of use in the case of organisms with fermentative powers of a weak and slowly acting character. One would therefore like to make use of anaerobic conditions, but the various forms of apparatus designed up to the present are so cumbersome that the trouble involved in their use outweighs the gain due to anaerobiosis. Besides, if an organism which ferments, say lactose, does give a colourless colony on bile salt lactose agar it is easy to subculture into a lactose broth tube in which, if freshly steamed, we have sufficiently anaerobic conditions to clear up any doubtful point.

Of course if one desires to compare with regard to the production of acidity the growth of an organism in a broth tube with the growth of the same organism on the surface of an agar plate anaerobic conditions are essential, as in the presence of oxygen one might get evidence of acid formation in the broth tube and none on the plate.

It will have been noted that the sugars, etc., are not added to the agar until just before use. The object of this procedure is to allow that kind of agar to be used which is most suitable to the case in question. By using a lactose agar the lactose fermenters, may be separated from the non-lactose fermenters. A mannit agar will pick out the mannit fermenters; a dulcit agar the dulcit fermenters, and so on. By combining two or more sugars, etc., in one agar we can exclude the organisms which attack these substances and leave only the colourless colonies for investigation. The use of a combination of several fermentable substances in one agar, suggested by me in 1905 (p. 335), has been adopted by Dr Houston (1907, p. 45).

With the idea of stimulating the growth of *B. typhosus* I have tried the effect of adding various substances to bile salt agar but without success. Urea, asparagin, nutrose, somatose, roborat, plasmon, and Nährstoff Heyden were without effect.

Soon after Loeffler published his method I investigated the effect of the addition of malachite green (Höchst 120) in varying proportions to bile salt agar and came to the conclusion that this medium could not be relied upon to give satisfactory results.

Encouraging results have been obtained by combining caffein (Roth's method) with bile salt agar when the amount of caffein was 0.33%. But up to the present this combination has not proved satisfactory enough to induce me to recommend it for general use.

The method which has given me the best result in the isolation of lactose fermenters is as follows:

The material to be examined is inoculated, either diluted or undiluted, into bile salt lactose broth. In the case of a water, the amounts taken are 250 c.c., 100 c.c., 10 c.c., 1 c.c., 0.1 c.c. and 0.01 c.c.

Concentrated bile salt broth is added to the 250 c.c., and 100 c.c.; ordinary broth is added to the 10 c.c.; and the other quantities are added to the bile salt broth in tubes.

After 18—24 hours' incubation at 37° C. the highest dilution which shows any change is picked out and surface cultures are made from it on bile salt lactose agar. The unchanged tubes etc., together with the plates, are put in the incubator and examined next day. If any change in the direction of acidity has taken place in any of the tubes, plates are made from them.

If on examining the plates all the colonies appear alike, then only three colonies are subcultured; but if more than one kind of colony is present then two colonies of each variety are subcultured. I have repeatedly tried to distinguish one organism from another by the appearance of the colonies on bile salt neutral-red lactose agar but invariably without success. On the same plate an organism may give irregular filmy "coli-like" red colonies and also round raised "aerogenes-like" red colonies. The form of a colony on bile salt agar *is of no value in identifying a lactose fermenting organism.*

If bile salt agar plates inoculated with a mixture of lactose fermenters are left upside down at room temperature a peculiar change occurs in some of the colonies. They take on a mucoid character and increase so much in depth that portions of the growth may drop down on to the cover of the plate.

The best medium to use for making cultures from the colonies is slant agar; and the growth is rubbed over the whole of the surface of the medium. After 18—24 hours' incubation at 37° C. subcultures are made from the agar into the various media; a good loopful of culture

being put into each tube. The sloped agar is used in order to get a sufficiently large quantity of growth and a large loop is used for the inoculations, because the mass of growth used for inoculation seems to have an effect in determining the rapidity of fermentation. By this means results are often obtained in 48 hours which otherwise might not be obtained under a week.

The media used are:—

1. *Glucose broth*—for Voges and Proskauer's reaction¹.
2. *Peptone water*—for indole.

This test should be performed after seven days' incubation; four days is not always enough even when using the Benzaldehyde reaction. If preferred, the peptone water may be omitted and the test performed upon an alcoholic extract of the agar growth (cf. Böhme, 1905).

3. *Litmus milk*—up to the present I have always made use of litmus milk. The experience thus gained has forced me to the conclusion that the litmus milk test is not necessary in the case of lactose fermenters.

4. *Gelatin*—I do not think much weight can be attached to the appearance of growths on gelatin of those organisms which grow on bile salt media. I use gelatin simply for the purpose of observing the presence or absence of liquefaction. Liquefaction may take place rapidly or slowly. Some organisms take 6—9 months to liquefy half an inch of gelatin (e.g. *B. oxytocus perniciosus*). By appropriate means the time may be shortened somewhat (MacConkey, 1906), but even then liquefaction may require a month to be complete. Retardation of solidification after being melted may be noticeable 2—3 weeks before the completion of liquefaction. Now in routine work it is quite impossible to wait longer than about two weeks. So in practice we are at present forced to class with the non-liquefiers any organism which does not liquefy gelatin in a fortnight.

5. *Bile salt broths*—containing respectively lactose, cane-sugar, dulcitol, adonitol and inulin².

After inoculation, the tubes containing these media are kept under observation as long as there is no change in the reaction of the medium³. As long as the medium remains neutral there is a possibility of the

¹ Recently I have isolated organisms which sometimes gave this reaction and sometimes did not do so. It may therefore be necessary to modify one's opinion with regard to this test.

² Inositol also has a value for differentiation purposes.

³ A week or 10 days is long enough in practice.

production of acid, but when once the reaction of alkalinity is visible in the inner tube the tubes may be discarded as acid production will not occur subsequently.

Gas may not be apparent for two or three days after the medium has turned acid.

Examinations are also made as to the presence or absence of motility and as to whether the organisms are Gram-positive or Gram-negative.

If the liquid media are inoculated in the morning, say between 10 and 11 A.M., they may be examined for motility the same afternoon between 4 and 5 P.M. It is not necessary to have visible growth for this purpose. A tube in which no growth is apparent to the naked eye may be fairly full of bacilli. To my mind the best idea of motility is gained by using a low power objective (half an inch), an eye-piece magnifying 8—10 times and dark ground illumination. This arrangement gives excellent results also in the case of agglutination reactions.

In the early stages of working with bile salt agar the results were somewhat conflicting and I gained the impression that this medium exercised a certain amount of inhibitory action on the growth of *B. coli communis* and *B. typhosus*.

As experience increased however it became apparent that, while some inhibitory effect might be exerted on organisms which had been grown on artificial media for a long time, freshly isolated organisms grew well.

As regards bile salt broth: Forster (1905) tested it against Drigalski and Conradi's nutrose agar. He used dilutions of a very thin emulsion of horse dung in sterile water for the inoculating material, with the following result:—

Nutrose agar aerobically at 37° C. for 24 hours.		MacConkey's medium anaerobically at 42° C. for 24 hours.	
Tube 1=0·1 c.c.	(growth)	Tube 1=0·1 c.c.	(growth)
2=0·01 c.c.	„	2=0·01 c.c.	„
3=0·001 c.c.	„	3=0·001 c.c.	„
4=0·0001 c.c.	„	4=0·0001 c.c.	„
5=0·00001 c.c.	„	5=0·00001 c.c.	„
6=0·000001 c.c.	(sterile)	6=0·000001 c.c.	(sterile)
7=0·0000001 c.c.	„	7=0·0000001 c.c.	„
8=0·00000001 c.c.	„	8=0·00000001 c.c.	„

By subculture *B. coli* was isolated from tube 5 of both series.

Thresh and Sowden (1904) compared phenol broth with bile salt broth in the examination of waters. They found that they obtained a

positive reaction in bile salt broth from a quantity of water which yielded no growth in phenol broth.

The following are experiments made by myself.

(I) A 1 in 100 emulsion of horse and calf faeces was put into a sterile Berkefeld filter (F 3) which was suspended in a jar of tap water. The water in the jar was changed every day. At the end of 12 weeks the contents of the filter were thoroughly mixed and a small quantity removed for examination. Dilutions were made up to 1 in 1000 millions. One cubic centimetre of each dilution from 1/1000 upwards was put into bile salt lactose broth and the same quantity also into ordinary alkaline nutrient bouillon. The tubes were incubated at 37° C. with the following result:—

Bile salt lactose bouillon 24 hours at 37° C.		Alkaline nutrient bouillon 24 hours at 37° C.	
Tube 1 = 1/1,000	A + G	Tube 1 = 1/1,000	Growth
2 = 1/10,000	A + G	2 = 1/10,000	„
3 = 1/100,000	A + G	3 = 1/100,000	„
4 = 1/1,000,000	No growth	4 = 1/1,000,000	No growth
5 = 1/10,000,000	„	5 = 1/10,000,000	„
6 = 1/100,000,000	„	6 = 1/100,000,000	„
7 = 1/1,000,000,000	„	7 = 1/100,000,000	„
48 hours at 37° C.		48 hours at 37° C.	
Tube 4	Growth, no acid	Tube 4	Growth
5	No growth	5	No growth
6	„	6	„
7	„	7	„

A = acid, G = gas.

Further investigation showed that there were lactose fermenters present in both kinds of broth in all dilutions up to 1/100,000 but not beyond; and that organisms were present in both media in the 1/1,000,000 dilution but there was no growth in any higher dilution.

In this case bile salt lactose broth proved just as good a medium as ordinary alkaline nutrient bouillon.

(II) On another occasion a similar experiment was performed with two other filters (F 4 and F 5) each containing a 1 in 100 emulsion of human faeces. After two months in tap water the contents were examined in exactly the same manner as in the case of F 3. The results were:—

F 4.

Bile salt lactose broth 24 hours at 37° C.		Alkaline nutrient bouillon 24 hours at 37° C.	
Tube 1 = 1/1,000	A + G	Tube 1 = 1/1,000	Growth
2 = 1/10,000	A + G	2 = 1/10,000	"
3 = 1/100,000	A	3 = 1/100,000	"
4 = 1/1,000,000	A	4 = 1/1,000,000	"
5 = 1/10,000,000	Nil	5 = 1/10,000,000	Nil
6 = 1/100,000,000	"	6 = 1/100,000,000	"
7 = 1/1,000,000,000	"	7 = 1/1,000,000,000	"
48 hours at 37° C.		48 hours at 37° C.	
Tube 3 = 1/100,000	A + G	Tube 3 = 1/100,000	Growth
4 = 1/1,000,000	A	4 = 1/1,000,000	"
5 = 1/10,000,000	? growth	5 = 1/10,000,000	"
6 = 1/100,000,000	"	6 = 1/100,000,000	? Growth
7 = 1/1,000,000,000	"	7 = 1/1,000,000,000	"

A = acid, G = gas.

Further investigation proved that there was growth in all tubes up to 1/10,000,000 but not beyond; and that lactose fermenters were present only up to 1/1,000,000.

F 5.

Bile salt lactose broth 24 hours at 37° C.		Alkaline bouillon 24 hours at 37° C.	
Tube 1 = 1/1,000	A + G	Tube 1 = 1/1,000	Growth
2 = 1/10,000	A + G	2 = 1/10,000	"
3 = 1/100,000	A + G	3 = 1/100,000	"
4 = 1/1,000,000	A + G	4 = 1/1,000,000	"
5 = 1/10,000,000	A	5 = 1/10,000,000	"
6 = 1/100,000,000	A	6 = 1/100,000,000	Nil
7 = 1/1,000,000,000	Nil	7 = 1/1,000,000,000	"
48 hours at 37° C.		48 hours at 37° C.	
Tube 5 = 1/10,000,000	A + fluorescence	Tube 5 = 1/10,000,000	Growth
6 = 1/100,000,000	A + G	6 = 1/100,000,000	"
7 = 1/1,000,000,000	?	7 = 1/1,000,000,000	?

A = acid, G = gas.

Subsequent inoculations showed growth in bile salt lactose broth up to 1/1,000,000,000 and in alkaline bouillon up to 1/100,000,000; but lactose fermenters were present only up to 1/100,000,000 in bile salt broth and up to 1/10,000,000 in alkaline bouillon.

The results of the examination of the contents of filters 4 and 5 after a sojourn of two months in tap water confirm previous experience and prove that bile salt broth is quite as good as any other medium for water analysis.

Bile salt in a proportion of 0·5% has no inhibiting effect upon *B. typhosus*, *B. coli communis* and allied organisms. As a matter of fact these bacilli will multiply in a broth containing a much larger percentage of bile salt. I have kept *B. typhosus*, etc. in 5% bile salt broth at room temperature for six weeks, and at the end of that time found them alive and capable of giving an abundant growth when re-inoculated on to agar. Of course there were no fermentable substances in the bile salt broth of this particular strength as the production of acid would have vitiated the experiment.

I have also tried the effect of increasing the quantity of bile salt in bile salt agar. The percentages used were 4, 5, 6, 7, 8 and 9%, and some 30 known organisms were tested. The results may be tabulated thus:—

<i>B. prodigiosus</i>	}	did not grow.
<i>B. mesentericus fuscus</i>		
<i>B. mesentericus vulgatus</i>		
<i>B. cavicida</i> (Brieger)		
		Growth in 4% in 48 hrs.
		„ „ 6% „ 4 days.
<i>B. oxytocus perniciosus</i>		„ „ „ „ „
<i>B. lactis aerogenes</i>	}	Poor growth on 9% in 48 hrs.
<i>B. capsulatus</i> (Pfeiffer)		
<i>B. cloacae</i>		
<i>B. typhosus</i>		
<i>B. coli communis</i> (Escherich)	}	Appeared to be unaffected except that some members of the Gaertner group (e.g. Hog Cholera, Arkansas—Smith) grew slowly during the first 24 hours.
<i>B. acidi lactici</i> (Hüppe)		
<i>B. neapolitanus</i>		
<i>B. pneumoniae</i> (Friedländer)		
<i>B. levans</i>		
<i>B. enteritidis</i> (Gaertner)		
<i>B. paratyphosus</i>		
etc., etc.		

The following organisms will not grow on ordinary bile salt media.

B. anthracis,
B. anthracoides,
B. diphtheriae,
B. xerosis,
B. subtilis,
M. tetragenus,

B. faecalis alkaligenes grows well on bile salt media that have been made strongly alkaline; but not on the ordinary media.

Streptococci vary very much, some grow well but slowly, others scarcely at all.

SUMMARY.

Bile salt media have been in use since 1900, and during the seven years which have elapsed it has been shown that *B. typhosus*, *B. enteritidis* (Gaertner), *B. coli communis* and similar organisms grow on these media just as well as on any other nutrient media. At 37° C. the growth of most of the organisms of the air and soil is inhibited by bile salt media.

These media can therefore be used with confidence for the isolation of *B. typhosus* and other intestinal organisms.

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ON THE RELATIONSHIP BETWEEN *BACILLUS PESTIS* AND *BACILLUS PSEUDOTUBERCULOSIS RODENTII* (PFEIFFER).

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THE *Bacillus pseudotuberculosis rodentium* (Pfeiffer) is the organism which morphologically and culturally most closely resembles *Bacillus pestis*. Galli-Valerio (1903) concluded that these bacilli resemble each other very closely in most respects but that *B. pseudotuberculosis* coagulates milk while the *B. pestis* does not; and while the *B. pseudotuberculosis* is not pathogenic for rats, it is extremely so for guinea-pigs, which succumb to cutaneous, nasal and conjunctival inoculations.

Zlatogoroff (1904) from a comparative study of 2 strains of the *B. pseudotuberculosis* and 22 strains of *B. pestis* (2 from rats and 20 from men) expresses the opinion that morphologically and culturally these organisms are practically identical; though *B. pseudotuberculosis* does not show such extensive pleomorphism as *B. pestis* and is non-virulent for rats and pigeons and only slightly so for white mice. The agglutination titre of the pest serum he tested did not exceed 1 in 500 and "clumping" took place quite as well in the case of *B. pseudotuberculosis* as in that of *B. pestis*. He obtained precipitin reactions in pest serum only with pest filtrates. Pest serum did not protect against infection with the *B. pseudotuberculosis* and *vice versa*. He failed entirely in his attempts to produce cross immunisation.

I have had at my disposal for study 11 strains of *B. pestis* and 7 of *B. pseudotuberculosis rodentium* (Pfeiffer) of various origins. The convention Ψ T.R. is used to designate the pseudotubercle bacillus.

When grown on agar or gelatine or in neutral bouillon no constant difference could be made out as regards the appearance of the growths or the morphology and staining reactions of the bacilli. The growth of

B. pestis on agar is usually found to be of a stringy consistency when touched with a needle but this is not a constant feature (cf. Shibayama, 1905). Not infrequently it is short and granular like that of the Bac. Ψ T.R. On the other hand none of my strains of Ψ T.R. ever gave a viscid growth; and they almost always grew more abundantly than *B. pestis*.

In litmus milk all the 18 cultures both of pest and pseudotuberculosis produced no change for three to four days at 35° C. Then in the case of the Ψ T.R. cultures the medium began to turn alkaline and finally became deep blue. The pest cultures were kept at 35° C. for about a week and then at room temperature for five weeks. At the end of this time there was no change apparent in the medium. On subculture an abundant growth of pest was obtained. In bile salt media containing various fermentable substances the strains of each gave the same reactions, and in this connection it is worthy of note that the ages of these strains varied from a few weeks to ten years. Acid but no gas is produced from glucose, mannite, laevulose, galactose and dextrin, while lactose, cane sugar, raffinose, sorbit, dulcit, adonit, inulin, amygdalin and α -methyl glucoside are unaffected (cf. MacConkey, 1905, p. 350). Vourloud (1907) obtained slightly different fermentation reactions, but he used ordinary nutrient agar and a trace of muscle sugar may have been present.

All these races were inoculated on to salt agar (NaCl 3%). At the end of 24 hours' incubation at 30° C. there was nothing to choose between them in the appearance of the growths. The growth of Ψ T.R. might have been slightly more abundant but that was all. Microscopically one could pick out a strain of pest which differed somewhat from some one particular strain of Ψ T.R.; but taking all the cultures together there was no bacillary form appearing among the films of pest which was not represented among those of Ψ T.R. also. After 48 hours' growth at 30° C. the majority of the cultures of Ψ T.R. showed a more abundant growth than the majority of the cultures of pest, but still there were some of the cultures of Ψ T.R. which had only grown slightly, while one culture of pest (10 years old) had grown more vigorously than any of the strains of Ψ T.R. Microscopically the relative appearances were the same as at 24 hours. The general impression gathered from a microscopical examination of all these cultures was that the yeast-like involution forms were not so abundant in the cultures of Ψ T.R., though one culture of Ψ T.R. was full of such forms, in fact fuller than any of the pest cultures.

We find then culturally only two points of difference between these

bacilli, namely the frequently viscid character of the growth of pest on agar and the development of an alkaline reaction in tubes of litmus milk inoculated with the Bac. Ψ T.R. These results are quite in accordance with those of Zlatogoroff, and as far as one can form an opinion from cultural characters one is constrained to recognise a close resemblance between these bacilli.

The results of precipitin tests emphasize this relationship. On the addition of pest filtrate to pest serum a cloudiness appeared almost at once and an abundant precipitate was deposited at the end of three hours at 37° C. When Ψ T.R. filtrate was added to pest serum these changes did not take place so rapidly. After three hours at 37° C. there was a distinct precipitate suspended in the serum but no deposit at the bottom of the tube. After three hours at 37° C. the tubes were placed in the cold room overnight. The precipitate caused by Ψ T.R. serum was not completely deposited at the end of 24 hours. On the other hand the pest filtrate precipitate was completely deposited and the supernatant fluid was quite clear.

The filtrates used were those employed to immunise guinea-pigs in the experiments on cross-immunisation detailed later.

Cross-immunisation.

Guinea-pigs are so very susceptible to infection by the Bac. Ψ T.R. that it is not to be wondered at if, in trying to immunise them with this bacillus, the mortality is considerable.

It is necessary to proceed very slowly with the process of immunisation and a month is a good interval to place between the injections. If the inoculations are not properly spaced the mortality is too high. Even when one proceeds with the greatest care one cannot count upon keeping all the animals alive until the test inoculation.

This state of affairs obtains also in the case of rats, though rats are said to be immune to Ψ T.R. It is true that they do not succumb to an acute disease but they do not all remain in as good health as before the inoculations. They waste and seem to become more susceptible to the attacks of other organisms. For instance in one experiment 12 white rats received killed cultures of the Bac. Ψ T.R. mixed with their food, about 9 c.c. of a 24-hour broth culture each day. Ten of these 12 rats died and from five of these ten I isolated a bacillus belonging to the group of the *B. enteritidis* (Gaertner). During this time there was not a single death among our stock rats.

The experiments detailed below show that animals which have withstood the process of immunisation with the Bac. Ψ T.R. are in most cases insusceptible to subsequent infection with pest.

EXPERIMENT I.

Four guinea-pigs were inoculated subcutaneously first with killed and then with living cultures of the Bac. Ψ T.R. Two of these animals died after the second dose. The remaining two received a third injection and then two months later were inoculated subcutaneously with pest, one control being infected at the same time. The pest inoculations were performed by Captain S. R. Douglas, I.M.S. The control animal died of pest on the fifth day while the other animals showed no signs of illness. Two months later they were killed and examined. One showed no signs of disease whatever. In the other there was a caseous inguinal gland from which no growth could be obtained. A film made from the caseous material showed appearances which might have been bacilli.

EXPERIMENT II.

In this experiment two series of animals were used.

Series I. Twelve guinea-pigs were inoculated subcutaneously with a quantity of killed emulsion equal to $\frac{1}{40}$ of an agar culture of Bac. Ψ T.R. Subsequently it was found that five does were pregnant. These were not used any more. Thirty-three days after the first injection a second inoculation of killed emulsion was given. This dose equalled $\frac{1}{10}$ of an agar culture. Thirty-seven days later each pig received killed emulsion equal to $\frac{1}{5}$ of an agar culture. Thirty-eight days later 1 c.c. of a living horse serum bouillon culture was given. Three pigs died after this dose. Forty-six days later the remaining four animals were inoculated with pest and found to be immune.

Series II. Twelve guinea-pigs were inoculated subcutaneously with killed emulsion of Bac. Ψ T.R., the first six receiving a dose equal to $\frac{1}{20}$ and the remainder a dose equal to $\frac{1}{10}$ of a 2-day agar culture. One of the second six died in three weeks. Thirty-eight days after the first injection each animal received subcutaneously 1 c.c. of a 5-day horse serum bouillon living culture of the Bac. Ψ T.R. Forty-six days later they received a test inoculation of pest at the same time as the animals in Series I and 10 controls.

The animals in Series II, like those in Series I, were found to be immune.

Of the controls seven died within a week and the *B. pestis* was isolated from every one. The remaining three were alive and well seven weeks later.

The result may be summarised thus :

Controls (10) mortality 70 %.

Immunised (15) mortality 0 %.

EXPERIMENT III. *To test the duration of the immunity.*

Ten guinea-pigs were inoculated subcutaneously with an emulsion of Bac. Ψ T.R. which had been killed by heat. Each animal received an amount of emulsion equal to $\frac{1}{20}$ of an agar tube.

Another series of 10 animals was treated in precisely the same manner except that an emulsion of another strain of Bac. Ψ T.R. was used.

Four of the first series and one of the second series died during the following 11 days.

Thirty-two days after the first injection a second dose was given subcutaneously, the quantity being equal to $\frac{1}{10}$ of an agar tube. A pig of the second series died after this inoculation.

After an interval of twenty-two days the animals received a third injection—the dose being 1 c.c. of a 24-hour living broth culture.

The animals were then kept for 204 days, i.e. nearly seven months, and were then given a test inoculation of pest; seven controls being inoculated at the same time.

The result was that six of the seven controls died of pest within eight days, three of the second series of immunised animals died on the seventh, eleventh, and twelfth days respectively, while one control and 11 immunised pigs remained alive and healthy.

Summarised result :

Controls (7) mortality 85·7 %.

Immunised (14) mortality 11·5 %.

It would appear then that in the majority of cases the immunity lasts at least six months.

EXPERIMENT IV. *To ascertain whether protection can be conferred by means of filtrates from cultures of Ψ T.R. bacilli.*

A Roux bottle of nutrient agar was inoculated with Ψ T.R. and incubated for three days at 37° C., when the growth was washed off the agar and emulsified in 200 c.c. of NaCl (0·85 %) solution. The emulsion

was heated in a flask for an hour at 60° C., a few drops of chloroform added, the flask plugged with a rubber cork and autolysis allowed to proceed for eight days at 37° C. The contents of the flask were then filtered through a Berkefeld filter, thus giving "ΨT.R. filtrate."

An exactly similar procedure was carried out with a strain of pest, and "pest-filtrate" obtained.

Ten guinea-pigs were inoculated subcutaneously with 1 c.c. of pest filtrate and another 10 pigs with 1 c.c. of ΨT.R. filtrate. One pest pig and two ΨT.R. pigs died five days later.

After an interval of 14 days all the animals were re-inoculated, the dose being 2 c.c. of filtrate. Two pigs of each series died during the subsequent three weeks.

One month after the second injection the animals received each 5 c.c. of filtrate.

Thirty-seven days later two pest pigs, two ΨT.R. pigs and two controls received a test inoculation of pest.

The result was that both controls died of pest within eight days, one pest pig died on the tenth day, and the remaining pest pig and both the ΨT.R. pigs remained alive and well.

The remaining animals were kept to be tested later.

At the end of four months it was found that the virulence of the pest bacillus used for testing had greatly diminished and that a test could not be carried out. So each pig received 1 c.c. of filtrate subcutaneously to keep up the immunity.

Four months after this injection these animals and six controls received a test inoculation of pest.

As a result four out of five pest pigs, one out of three ΨT.R. pigs and five out of six controls died of pest.

The results of both parts of this experiment may be taken together and summarised thus:

Controls (8) mortality 87·5 %.

Pest immunised (7) mortality 71·5 %.

ΨT.R. immunised (5) mortality 20 %.

These results show that immunity to pest can be conferred by means of filtrates of cultures of the ΨT.R. bacillus and that this immunity lasts several months.

So far the experiments had been confined to guinea-pigs, animals which are acknowledged to be very susceptible both to pest and to ΨT.R. The question then naturally arose as to whether rats could be similarly

protected by inoculations of the bacillus Ψ T.R., an organism to which they are more or less immune.

EXPERIMENT V.

Two series of 10 rats each were inoculated subcutaneously with 0.5 c.c. of a living 24-hour broth culture of the Bac. Ψ T.R., a different strain being used for each series. Seven weeks later they received 1 c.c. of a 24-hour living broth culture subcutaneously.

Seven animals died during immunisation.

One hundred and sixty (160) days after the second immunising injection Dr C. J. Martin kindly tested these rats with pest. Nine of the one series, four of the other series and five controls were inoculated at the same time. The result was that all the controls were dead of pest on the fourth day. Of the immunised animals three died on the fourth day, one on the fifth day and one on the seventh day. The remaining eight remained alive and healthy.

Thus of the

Controls (5) mortality 100 %

Protected (13) mortality 38.5 %

when tested five months after the last immunising injection.

SUMMARY.

(1) Morphologically and culturally the *Bacillus pseudotuberculosis rodentium* (Pfeiffer) bears a strong resemblance to *B. pestis*.

(2) The filtrate from an autolysed agar culture of *B. pseudotuberculosis rodentium* (Pfeiffer) and a similar filtrate from a *B. pestis* culture both gave a precipitin reaction with pest serum.

(3) It has been found possible to immunise both guinea-pigs and rats against plague by means of inoculations of cultures of *B. pseudotuberculosis rodentium* (Pfeiffer), and this immunity lasted in many cases at least six months.

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THE PREVENTION OF COMPRESSED-AIR ILLNESS.

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(From the Lister Institute of Preventive Medicine.)

[With 7 Figures and 3 Plates.]

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INTRODUCTION.

MEN who have been working in compressed air, as in diving, preparing foundations of bridges, etc. under water, or making tunnels or shafts through water-bearing strata, are liable on their return to atmospheric pressure to a variety of symptoms generally known as "diver's palsy" or "caisson disease," but which may more conveniently be designated "compressed-air illness." It was shown experimentally by Paul Bert¹ that these symptoms are due to the fact that gas (chiefly nitrogen) which goes into solution in the blood and tissues during exposure to compressed air is liberated in the form of bubbles on too rapid decompression, and produces local or general blockage of the circulation or other injury. Subsequent investigations, for an account of which we must refer more particularly to the treatise on the subject by Heller, Mager and v. Schrötter² and to recent papers by Hill and McLeod³ and Hill and Greenwood⁴, have confirmed and extended Paul Bert's conclusions.

It was pointed out by Paul Bert that by means of very slow decompression the symptoms of caisson disease could be avoided, but his experiments were not sufficient to furnish data as to what rate of decompression would be safe. Nor has subsequent human experience in engineering undertakings solved this problem; and the risks attending work in compressed air at excess pressures of over $1\frac{1}{2}$ to 2 atmospheres are notorious. Heller, Mager and v. Schrötter have endeavoured to formulate rules as to safe decompression; and they express the belief that perfectly uniform decompression at the rate of 20 minutes an atmosphere would always be safe. Following this rule, which is based on a calculation, Hill and Greenwood decompressed themselves, without any serious symptoms, after short exposures at excess pressures of as much as five and even six atmospheres.

Although the rules formulated by the above-mentioned observers constituted a distinct step in advance, it appeared to us that, for reasons which will be explained below, there were grave doubts as

¹ *La Pression Barometrique*, 1878.

² *Luftdruckerkrankungen*, 1900; also v. Schrötter, *Der Sauerstoff in der Prophylaxie und Therapie der Luftdruckerkrankungen*, 2nd edition, 1906. The former work contains a very full abstract of all previous investigations on the subject.

³ This *Journal*, vol. III. (1903), p. 401 (and references there given): see also *Recent Advances in Physiology*, 1906, pp. 233—255.

⁴ *Proceedings of the Royal Society*, vol. LXXVII. p. 442, 1906; vol. LXXIX. p. 21, 1907; also *British Medical Journal*, July 7th, 1906, Feb. 16th, 1907, June 22nd, 1907.

to the safety of their recommendations, and particularly as to whether uniform decompression is desirable. The need for framing definite rules as to safe decompression in the shortest possible time presented itself in a very definite form in connection with the work of the Admiralty Committee on Deep Diving¹, of which one of us was a member. Our investigation, which was planned with the more particular object of furnishing information required for securing the safety of divers ascending from deep water, was rendered possible by the gift to the Lister Institute by Dr Ludwig Mond, F.R.S. of a large experimental steel pressure chamber and by substantial financial and other help from the Admiralty, Messrs John Aird and Son, the late Mr Basil Ellis, Messrs S. Pearson and Son, Ltd., and Messrs Price and Reeves.

The formation of gas bubbles in the living body during or shortly after decompression evidently depends on the fact that the partial pressure of the gas or gases dissolved in the blood and tissues is in excess of the external pressure. But it is a well-known fact that liquids, and especially albuminous liquids such as blood, will hold gas for long periods in a state of supersaturation, provided the supersaturation does not exceed a certain limit. In order to decompress safely it is evidently necessary to prevent this limit being exceeded before the end of decompression. Whether or not the decompression is free from risk will depend on the degree of supersaturation which can be borne with safety, the extent to which the blood and tissues have had time or opportunity to become saturated, and the extent to which they have had time to become desaturated again during decompression. In carrying out our investigations we have kept these three factors constantly in view, and it is necessary to discuss them in some detail before proceeding further.

¹ The Report of this Committee, which has recently appeared as a blue-book, contains a full account of the experimental investigations on Diving, carried out under its auspices at Portsmouth, off the West Coast of Scotland, and elsewhere, during the last two years : also a short summary of the experiments detailed in the present paper, and many data as to the occurrence of compressed-air illness in connection with diving and other work in compressed air. The conclusions and recommendations of the Committee are summarised at the beginning of the Report.

PART I. THEORETICAL.

A. *The rate of saturation of the body with nitrogen during exposure to compressed air.*

When a man or animal is placed in compressed air, the blood passing through the lungs will undoubtedly take up in simple solution an amount of gas which will be increased above normal in proportion to the increase in partial pressure of each gas present in the alveolar air. The experiments of Haldane and Priestley¹, which have since been extended by Hill and Greenwood², show that the partial pressure of CO₂ in the alveolar air remains constant with a rise of atmospheric pressure: hence there can be no increase in the amount of CO₂ present in the blood during exposure to compressed air. As regards oxygen, the amount in simple solution in the arterial blood will certainly increase in proportion to the rise in alveolar oxygen pressure; but as soon as the blood reaches the tissues this extra dissolved oxygen, which (except with exposures to enormous pressures) is only a small part of the total available oxygen in the arterial blood, will be used up, so that in the tissues and venous blood there will be at most only a very slight increase in the partial pressure of oxygen. For practical purposes therefore we need only take into consideration the saturation of the body with nitrogen.

In view of what is known as to the ease and completeness with which the blood becomes aerated in its passage through the lungs, there seems no reason to doubt that in compressed air the blood reaching the lung capillaries must become instantly saturated with nitrogen at the partial pressure existing in the alveolar air (see p. 351). At the commencement of exposure to compressed air this blood, on being carried to the tissues, will by diffusion share with them its excess of nitrogen and then return to the lungs for a fresh charge. By the constant repetition of this process the tissues, and the venous blood leaving them, will gradually become more and more saturated with nitrogen at the partial pressure of the nitrogen in the alveolar air, which will be practically the same as in the inspired air. Since the rate of blood supply and the solubility of nitrogen per unit mass of tissue vary greatly in different parts of the body, the rate of saturation

¹ *Journal of Physiology*, vol. xxxii. (1905), p. 229.

² *Proc. Roy. Soc., B*, vol. lxxvii. p. 442.

will vary correspondingly. We may however form some rough general idea of the average rate of saturation by assuming as a basis of calculation that the blood is evenly distributed throughout the body, and that the tissues are similarly constituted in all parts.

According to the figures adopted by Bohr¹, 100 c.c. of blood take up in simple solution at the body temperature 0·87 c.c. of nitrogen for each atmosphere of air pressure. This is only 8 % less than would be taken up by water under the same conditions. Blood contains nearly the same percentage of solids as the semi-liquid tissues (apart from fat) in most parts of the body, and we may assume that these tissues will take up nearly the same proportion of nitrogen as blood. The earthy constituents of bone (about 3 % of the body weight) probably take up no nitrogen. On the other hand the body fat, as was recently shown by Vernon², who made a number of determinations at the body temperature with special reference to our investigations, takes up about six times as much nitrogen as an equal weight of blood. The body of a well-nourished man probably contains fully 15 % of its weight as fat or fatty material. Hence it may be estimated that it will, when saturated at any given pressure, on an average take up, weight for weight, about 70 % more nitrogen in simple solution than the blood under the same conditions, and that the whole body of a man weighing 70 kilos will take up about one litre of nitrogen for each atmosphere of excess pressure.

Now the weight of the blood in man is about 4·9 % of the body weight³: hence the amount of nitrogen held in solution in the body, when it is completely saturated at any given pressure, will be about $\frac{170}{4\cdot9}$, or 35 times as great as the amount present in the blood alone. If therefore the blood distributed itself evenly and at the same rate throughout the body, the latter would have received, at the end of one complete round of the blood after sudden exposure to high pressure of air, one thirty-fifth of the excess of nitrogen corresponding to complete saturation. The second round of the circulation would add one thirty-fifth of the remaining deficit in saturation, *i.e.* $\frac{1}{35} \times \frac{34}{35}$ of the total excess: the third round would add $\frac{1}{35} \times (\frac{34}{35} \times \frac{34}{35})$ of the total excess, and so on. On following out this calculation, it will be found that half the total excess of nitrogen would have entered the body

¹ *Nagel's Handbuch der Physiologie*, vol. I., 1905, p. 63.

² *Proc. Roy. Soc.*, vol. LXXIX. B, 1907, p. 366.

³ Haldane and Lorrain Smith, *Journal of Physiology*, vol. xxv., 1900, p. 340.

after 23 rounds of the circulation, three-fourths after 46 rounds, seven-eighths after 69 rounds, and so on. The progress of the saturation of the body with nitrogen is thus a logarithmic curve of the form shown in Figure 1¹.

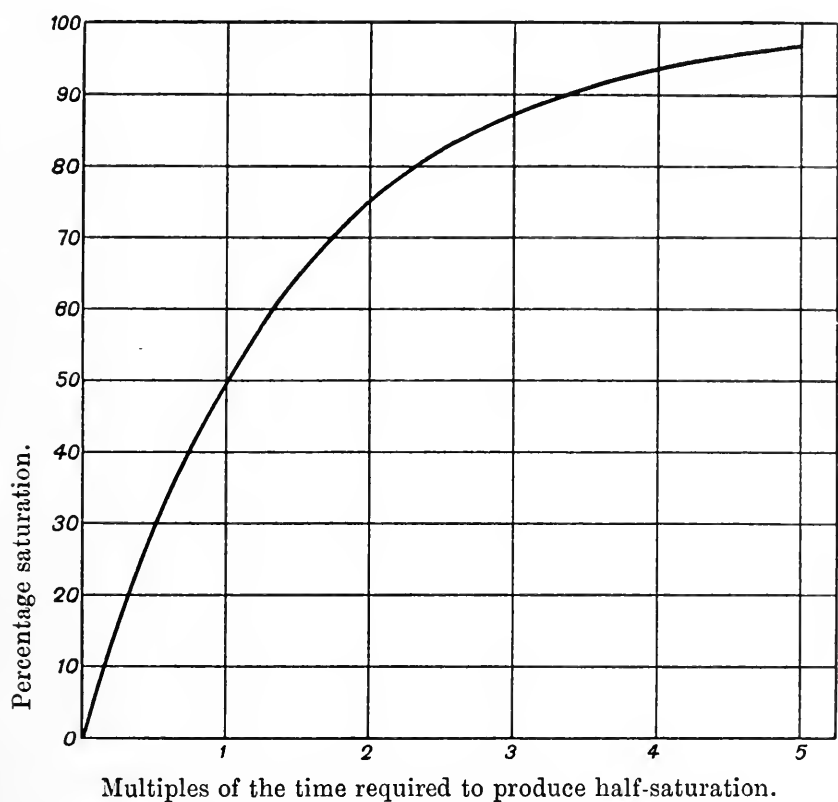


Fig. 1. Curve showing the progress of saturation of any part of the body with nitrogen after any given sudden rise of air pressure. The percentage saturation can be read off on the curve, provided the duration of exposure to the pressure, and the time required to produce half-saturation of the part in question, are both known. Thus a part which half-saturates in one hour would, as shown on the curve, be 30 % saturated in half-an-hour, or 94 % saturated in 4 hours.

Experiments on animals have shown that the venous blood entering the lungs contains about two-fifths less of oxygen than the arterial blood. If we assume that the same proportion holds good for a man at rest, and that very little oxygen is used up in the lungs themselves, the percentage of oxygen gained by the blood in the lungs must be about 8 %, or about double the percentage diminution in the expired

¹ This calculation is in principle similar to that made by Zuntz (*Fortschritte der Medizin*, 1897, No. 16), and worked out more fully by Heller, Mager and v. Schrötter (*loc. cit.*). On account, however, of the discovery that fat has a very high coefficient of absorption for nitrogen, and that the blood volume in man is considerably less than was formerly supposed, our calculation gives a much slower rate of saturation per round of the circulation.

air as compared with the inspired air. The volume of blood passing through the lungs is therefore about double the volume of air breathed. Since this volume of air (measured dry and at standard pressure and temperature) averages about seven litres per minute¹ for a man of 70 kilos during rest, the volume of blood passing through the lungs may be estimated at about 3·5 litres per minute². The total blood volume is however also about 3·5 litres, so that a volume of blood equal to the total blood volume probably passes through the lungs about once a minute during rest. We may therefore substitute minutes for rounds of the circulation in the above calculation of the rate of saturation of the body with nitrogen, so that, if the assumptions made for the purposes of the calculation held good for a man exposed to compressed air, his body would be half saturated with the excess of nitrogen in 23 minutes, three-fourths saturated in 46 minutes, etc.

In reality, however, this calculation affords at best only a very rough general idea of the actual rate of saturation, since it is known that the distribution of blood per unit of body weight through various parts of the body varies greatly, and that the rate of circulation through any given part varies according as the part is at rest or in a state of activity. The proportion of fat and fatty material is also very different in different parts of the body, so that the capacities of different tissues for taking up nitrogen must vary accordingly. We should expect therefore that some parts of the body will saturate much more rapidly than the calculation shows, and other parts much more slowly. Direct experimental evidence of far more rapid saturation in some parts of the body has recently been furnished by Hill and Greenwood³. Their method was to determine the free nitrogen in samples of urine secreted shortly after exposure to high pressure, and shortly after return to normal pressure. A sufficiently copious secretion of urine was produced by previously administering large drinks of water to the subject of the experiment; and they found that, within about ten minutes of exposure to high pressure, samples of urine secreted were saturated at this pressure. Conversely, on lowering the pressure to normal, the excess of nitrogen disappeared within a few minutes.

¹ Haldane and Priestley, *loc. cit.*, p. 245.

² As a result of numerous experiments on man with the lung catheter Loewy and v. Schrötter (*Untersuchungen über die Blutcirculation beim Menschen*, 1905, p. 90) infer that the average rate of blood flow during rest is slightly faster. At present, however, there is some doubt as to the interpretation of results obtained by the lung catheter method.

³ *Proc. Roy. Soc. B*, vol. LXXIX., p. 21, 1907.

These results seem to show conclusively that the kidney substance became saturated with nitrogen at a rate about ten times as great as would correspond to the above calculation. From the data given it appears, however, that urine was being secreted with great rapidity during the experiments. For instance, 135 c.c. were secreted in five minutes in one observation where the quantities and times are recorded. This is about thirty times the average rate of secretion, so that the circulation of blood through the actively working kidneys must have been greatly increased.

Equally clear evidence of the existence of a far slower rate of saturation is afforded by the experience of men working in compressed air, particularly in caissons and tunnels at moderate pressures. It is well known to those practically familiar with such work that the risk of symptoms occurring on decompression depends on the duration of the exposure. There is very little risk on rapid decompression after short exposures of less than an hour to an excess pressure of two atmospheres or even somewhat higher pressure; but as the duration of exposure increases hour by hour, so do the risks on decompression increase. We are assured by Mr E. W. Moir (of the firm of Messrs S. Pearson and Son, Ltd., Westminster), who has had an exceptionally large experience of tunnelling work in compressed air at excess pressures up to about $2\frac{1}{2}$ atmospheres, that the maximum of risk is not reached after even three hours, so that a limitation of working shifts to three hours markedly diminishes the frequency of compressed-air illness. Hence in some parts of the body saturation with nitrogen must still be incomplete after three hours. Another observation pointing in the same direction is that when the daily working period was $8\frac{1}{2}$ hours under pressure with two intervals of about $1\frac{1}{2}$ hours each for meals at ordinary atmospheric pressure, cases of caisson disease usually occurred after the last decompression in the evening and not when the men came out for meals¹.

Our own observations on animals afford fresh evidence bearing in the same direction. We found that in goats the risks on decompression increase with the length of exposure to pressure up to from two to three hours (see below, p. 396).

In different warm-blooded animals the rate of respiratory exchange varies, roughly speaking, according to the ratio of body surface to weight. The smaller the animals, therefore, the greater is the respiratory exchange per unit of body weight, and the more rapid must be the

¹ G. W. M. Boycott, *Trans. Inst. of Civil Engineers*, vol. CLXV., 1906.

circulation. In consequence small animals, when placed in compressed air, must saturate their tissues more rapidly in proportion to their more active respiratory exchange; and, conversely, they will free themselves more rapidly, during or after decompression, from the excess of nitrogen. Hence results obtained with small animals as to the time required for complete saturation, or for safe decompression, are not directly applicable to man. We selected goats for our experiments as they were the largest animals which could be conveniently used; but their weights averaged only about one-fourth to one-third of the weight of an adult man. As the surfaces of different mammals are roughly as the cube roots squared of their weights, we should expect that in goats of this size the respiratory exchange per kilo of body weight would be about two-thirds greater than in man. Direct determinations showed that this was the case (see p. 381). Hence if it required three hours exposure to a high pressure to effect practically complete saturation¹ of the more slowly saturating tissues of a goat with nitrogen, about five hours would be required for a man. An inspection of Fig. 1 (p. 347) will show that if these tissues became 50 % saturated in about 45 minutes in goats and 75 minutes in man, they would be 94 % saturated in three hours for goats, and in five hours for man. A higher degree of saturation than this would scarcely be appreciable, and we have concluded that for practical purposes any slower rate of saturation than this, and correspondingly slower rate of desaturation, need not be allowed for, unless the percentage of fat in the body is abnormally high. We must admit, however, that there is some evidence, both from our own experiments and from practical experience in work in compressed air, that in the parts of the body which are the seat of "bends" a still slower rate of saturation may exist.

B. *The rate of desaturation of the body with nitrogen during and after decompression.*

If the pressure is rapidly diminished to normal after exposure to saturation in compressed air, and no gas bubbles are liberated in the body, it is evident that for each part of the body the curve of desaturation will be similar to that of saturation, provided the physiological conditions are constant. The venous blood will give off practically the

¹ The only method apparently available to determine the time of complete saturation in normal animals is to subject them to a series of experiments in which the pressure and decompression are kept constant and the time of exposure varied, and to observe when the effects cease to become any worse. The method is open to obvious limitations.

whole of its excess of dissolved nitrogen during its passage through the lungs¹, and at each round of the circulation will bring back a fresh charge of nitrogen (at the partial pressure existing in the tissues) to be given off. The parts which become half desaturated by this process in a given time will be three-fourths desaturated in double the time, and so on. The slowest saturating tissues will thus, in accordance with our previous calculation, take one and a quarter hours to become half desaturated in man.

The normal combined gas pressure of nitrogen, oxygen and CO₂ in the tissues and venous blood may be estimated as about 90 % of an atmosphere, so that if the nitrogen pressure be more than an eighth above normal the total gas pressure will be above atmospheric pressure. Supposing therefore that before decompression the most slowly saturating parts of the body (*i.e.* those half saturating in one and a quarter hours) had been saturated to an excess pressure of two atmospheres of air, it would take about five hours at atmospheric pressure to reduce this excess pressure to a sixteenth (or an eighth of one atmosphere) and so bring down the total gas pressure in the parts in question to about atmospheric pressure. The slowness of desaturation must be as clearly borne in mind as the slowness of saturation, in connection with all the phenomena of compressed-air illness.

If gas bubbles are formed in consequence of too rapid decompression, they will naturally tend to increase in size by diffusion into them, in whatever part of the body they may be except the arteries, for some time after the end of decompression. They may thus easily cause blocking of small vessels, and even if they are carried to the right side of the heart or the pulmonary arteries, and lodge there, they will increase in bulk until the total gas pressure in the mixed venous blood falls to one atmosphere. The same remark applies to bubbles which

¹ In view of the enormous surface (probably more than 100 square metres) presented by the lung alveoli for diffusion it seems hardly possible to doubt that the blood during its passage through the lungs becomes saturated or desaturated to almost exactly the pressure of nitrogen in the alveolar air. According to the calculations of Loewy and Zuntz (*Die physiologischen Grundlagen der Sauerstoff-Therapie* in Michaelis' *Die Sauerstofftherapie*, Berlin, 1904), a difference in partial pressure of oxygen of less than 1 mm. of mercury would account for the diffusion of 250 c.c. of oxygen per minute through the alveolar walls. With a difference in partial pressure of nitrogen of two atmospheres, or 1520 mm. of mercury, between the blood and the alveolar air only about 70 c.c. of nitrogen would require to pass per minute in order to establish complete saturation, or desaturation, of the blood. The conditions are thus enormously more favourable for the taking up or giving off of this nitrogen than for the taking up of oxygen by diffusion during normal respiration.

lodge in the branches of the portal veins. If small bubbles are carried through the lung capillaries and pass, for instance, to a slowly desaturating part of the spinal cord, they will there increase in size and may produce serious blockage of the circulation or direct mechanical damage. Apart from this increase of size the air bubbles passing along the arteries are probably too small to cause any harm. Once formed they will under ordinary conditions take a long time to become re-absorbed, since even after the gas pressure in the blood and tissues has fallen to normal, the excess of nitrogen pressure in the bubbles over that in the blood and tissues will only be about a tenth of an atmosphere at most. In one case we found bubbles in the veins of an animal which died two days after suffering from severe decompression symptoms (see below p. 421).

In order to avoid the risk of bubbles being formed on decompression, it has hitherto been recommended that decompression should be slow and at as nearly a uniform rate throughout as possible. We must therefore carefully consider the process of desaturation of the body during slow and uniform decompression. For convenience in calculation we may imagine the process as occurring in a series of time-intervals, the first half of each of which is spent at the pressure existing at the beginning of the interval, and the second half at the pressure existing at the end. Let us suppose, for instance, that the body has been completely saturated with nitrogen at an excess pressure of five atmospheres of air, and that decompression occurs at a rate of one atmosphere in 20 minutes. The process may be divided into five periods of 20 minutes, during each of which the pressure falls one atmosphere. We can then easily calculate how far desaturation will have gone at the end of each period, and from these data construct a desaturation curve.

Let us first consider the mean desaturation rate of the whole body, assuming that, when the pressure is suddenly raised or diminished to a certain level, the tissues will on an average saturate or desaturate themselves by 50 % in 23 minutes, which was shown above to be a probable average rate. A reference to the curve (Fig. 1) shows that ten minutes' exposure to the reduced pressure of four atmospheres in excess will reduce the saturation by 28 % of the difference between five and four atmospheres, *i.e.* by 0.28 of an atmosphere. Hence at the end of 20 minutes the tissues will on an average be saturated to 4.72 atmospheres. Ten more minutes at four atmospheres will reduce the saturation to 4.5 atmospheres, and ten minutes at three atmospheres

will further reduce it by 28% of $4.5 - 3$, *i.e.* by 0.42 atmosphere. Hence at the end of the second twenty minutes the saturation of the tissues will be 4.08 atmospheres. Continuing this calculation we get the desaturation curve shown in Fig. 2, from which it will be seen that when atmospheric pressure is reached the tissues are still saturated to an excess pressure corresponding to 1.4 atmospheres of air.

Fig. 2 also shows a similar curve for the parts which saturate and desaturate most slowly, and which, according to our previous calculations, take one and a quarter hours to become half saturated. At the end of decompression these slowly desaturating parts, as shown on the curve, are still saturated to 3.15 atmospheres. This of course represents a most formidable excess; and, as will be shown below (p. 401), uniform decompression at this rate is dangerous even to goats, and would certainly be extremely dangerous to men, who desaturate a good deal more slowly than goats.

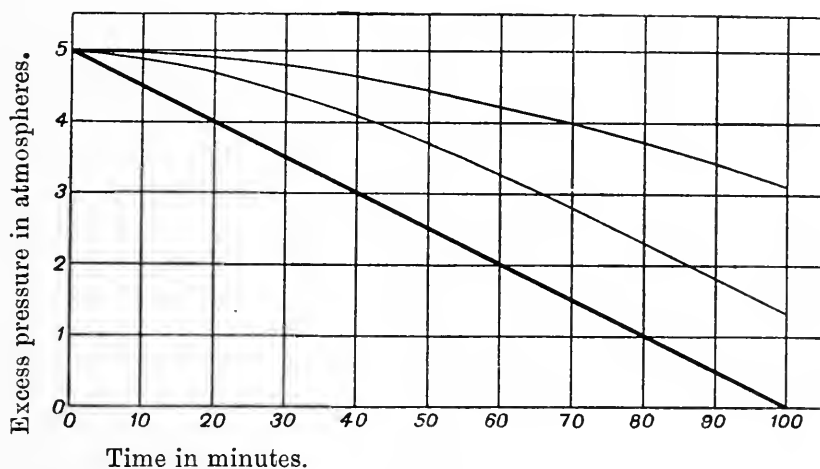


Fig. 2. Desaturation during uniform decompression after complete saturation at 5 atmospheres excess pressure. The thick line represents the air pressure: the upper and lower thin lines represent respectively the progress of desaturation in parts of the body which half saturate in 75 and 23 minutes.

Inspection of Fig. 2 shows that with uniform decompression the nitrogen pressure in the body lags behind that of the air, and that (in the case of the slowly desaturating parts) the amount of the lag increases during the whole time of a decompression lasting 100 minutes. No other result seems possible, and actual experiments point strongly in the same direction, as will be shown presently. We must emphatically dissent from the conclusion drawn by Heller, Mager and v. Schrötter that decompression at the uniform rate of 20 minutes an atmosphere prevents any dangerous retention of gas in the body. To

prevent a maximum lag of more than one atmosphere, it would be necessary to decompress at a rate of over one and a half hours an atmosphere if the decompression were uniform and from an excess pressure of five atmospheres¹.

The examples given will be sufficient to illustrate the extreme slowness with which desaturation must occur with a uniform rate of decompression. This slowness has never hitherto been recognised, but must evidently be reckoned with in devising measures for the prevention of caisson disease.

It is clear that the rate of desaturation might be hastened by either (1) increasing the difference in nitrogen pressure between the venous blood and the air in the lungs, or (2) increasing the rate of blood circulation. In either case the blood would give off through the lungs an increased amount of the excess of nitrogen in a given time.

In order to increase the difference in nitrogen pressure between the venous blood and the alveolar air it has been proposed to give a diver oxygen to breathe during, or before decompression. As long, however, as the pressure was above about one atmosphere in excess, or 15 lbs., it would be impossible to do this safely, since, as will be explained more fully below, the effects might be rapidly fatal owing to oxygen poisoning. The possible applications of oxygen are thus somewhat limited, while the complications involved would be very considerable. The same end can, however, be attained in another way, as will be shown in the following section.

The rate of blood circulation can be increased considerably by muscular exertion. Quite moderate exertion is sufficient to increase the respiratory exchange to three or four times the normal; and the rate of blood flow through the lungs must be increased to something approaching to a corresponding extent. Unfortunately, the increased blood flow is chiefly through the muscles which are working, but probably many parts of the body participate to a greater or less extent in the extra blood supply. Muscular work must correspondingly increase the rate of saturation of the body with nitrogen. For this reason it seems desirable that where work has been done in compressed air, so that the muscles and associated tissues have probably become rapidly saturated with nitrogen, there should also be muscular exertion

¹ It is evidently a mistake to assume that a given rate of uniform decompression, such as 20 minutes per atmosphere, is either necessary for safety in all cases, or would be actually safe except from some limit of pressure. From a pressure below this limit the rate will be unnecessarily slow, and from above it dangerously fast.

during decompression. The rate of desaturation will thus be increased so as to compensate for the increased rate of saturation. In the case of short exposures to compressed air, as in diving work, this is specially important. Even, however, when there has been no special muscular work in the compressed air movements of joints and massage of the skin etc. will probably hasten desaturation. This has been clearly pointed out by Hill and Greenwood¹.

Another method which can be employed for increasing the circulation in the case of divers is to restrict the air supply, so that the partial pressure of CO₂ in the air of the helmet may rise sufficiently to stimulate the respiration and circulation. Both methods are now used in the Royal Navy during the ascent of divers.

C. *The limits of safety in decompression.*

It is a fact well known to those practically acquainted with work in compressed air that even with very rapid decompression there is no risk of caisson disease unless the pressure has exceeded a certain amount. It seems perfectly clear that no symptoms occur with less than one atmosphere² of excess pressure, however long the exposure may be. Whether any distinct symptoms ever occur with less than about 1.25 atmospheres (18½ lbs. per square inch or 41 feet of sea water) seems very doubtful: at any rate they are very exceptional. At pressures a little above 1.25 atmospheres occasional slight cases begin to be observed, and their frequency and gravity rapidly increase with higher pressures unless the time of exposure is limited or slow decompression is resorted to. The lowest pressure at which we have been able to find any record of a death occurring from caisson disease is 23 lbs. or 1.6 atmospheres³. As will be seen below, we were able to obtain slight symptoms on rapid decompression in 1 out of 22 goats after long

¹ *Proc. Roy. Soc. B*, vol. LXXVII., p. 449, 1906.

² One atmosphere or 760 mm. of mercury = 14.7 lbs. per square inch, about 1 kilogram per square centimetre, 34 feet of fresh water, 33 feet of sea water. In this paper where pressures are defined in pounds or atmospheres without qualification, reference is intended to the excess over atmospheric pressure as shown on gauges, not to the absolute total pressure.

³ Babington and Cuthbert, *Dublin Quarterly Journal of Medical Sciences*, vol. XXXVI., 1863, p. 312. In the list of fatal cases given by Heller, Mager and v. Schrötter (*Luft-druckerkrankungen*, p. 1072), are entered two deaths at a pressure of 1.4 atmospheres. A perusal of Paul Bert's original account (*La Pression Barometrique*, p. 401) shows that both the pressure and the cause of death are quite uncertain.

exposure (four hours) to 1·36 atmospheres or 20 lbs. With 25 lbs. (1·7 atmospheres) two cases of slight illness occurred out of 23 animals.

If the risks of rapid decompression depended simply on the extent to which the blood and tissues are supersaturated with nitrogen on decompression, we should expect to find that even a short exposure to such an excess pressure as two atmospheres would be risky with rapid decompression: for there can be no doubt that within, say, half an hour or forty minutes the tissues, and the blood returning from them, must be for all practical purposes fully saturated in many parts of the body, and particularly in parts of great physiological importance which are richly supplied with blood. Nevertheless it seems to be well established that a man may stay without serious risk for forty minutes at a pressure which would involve great danger on rapid decompression if he remained in it for several hours.

Parts of the body with a rapid circulation will become very completely saturated in a comparatively short time, but the highly supersaturated blood which first returns from them on rapid decompression can remain but a very short time supersaturated during each round of the circulation, and on reaching the large veins will mix with less highly saturated blood from other parts of the body. It would seem that the state of high supersaturation in any portion of blood lasts for too short a time to enable bubbles to form.

If this interpretation of the facts is correct, we should expect to find with small animals, which rapidly saturate and desaturate, that a higher pressure would be required to produce symptoms on rapid decompression after a long exposure than in the case of larger animals. The general experience of previous observers is in accord with this, and our own experiments (see below p. 402) showed that we could produce no obvious effects in mice, and very few in rabbits, rats, and guinea-pigs, by sudden decompression after exposures at pressures which were invariably or frequently fatal to goats.

Since supersaturation to the extent of about 1·25 atmospheres above normal atmospheric pressure can be borne with impunity, though a greater degree of supersaturation is risky, it seems clear that, in decompressing after prolonged exposure to high pressures, the rate of decompression should be sufficiently slow to prevent any greater excess of saturation than this in any part of the body at the end of decompression. On the other hand decompression should evidently be as rapid as is possible, consistently with safety. A pressure of 1 to 1·25 atmospheres above normal corresponds to from 2 to 2·25 times the

normal atmospheric pressure; but the *volume* (not the *mass*) of gas (measured at the existing pressure) which would be liberated if the whole excess of gas present in supersaturation were given off is the same whether the absolute pressure is reduced from two to one atmospheres, or from four to two, or from eight to four. Hence it seemed probable that, if it is safe to decompress suddenly from two atmospheres of absolute pressure to one, it would be equally safe to decompress from four atmospheres absolute to two, from six atmospheres absolute to three, etc. Our experiments, which are detailed below (p. 398), have shown that this is the case¹. The process of desaturation can therefore be hastened very greatly by rapidly reducing the absolute pressure to half, and so arranging the rest of the decompression that the saturation in no part of the body shall ever be allowed to correspond to more than about double the air pressure. The main advantage of this plan is that the discharge of nitrogen from the tissues is from the outset of decompression increased to the greatest rate which is safe. The rate of discharge evidently depends on the difference in partial pressure of nitrogen between the venous blood and the alveolar air; and by keeping this difference at the maximum consistent with safety a great saving of time is effected. Detailed investigations have completely justified the adoption of this principle: they are described below, and comprise, besides a series of observations on animals, a number of experiments in which Lieut. Damant and Mr Catto were exposed to excess pressures up to 80 pounds, or 6·4 atmospheres of absolute pressure, in the experimental chamber and to 93½ pounds, or 7·4 atmospheres, in actual diving. The method greatly simplifies the problem of safe decompression, and gets rid of many practical difficulties, particularly in connection with deep diving. It may be conveniently referred to as the method of "stage decompression," and is so described in the sequel, though its essential peculiarity does not lie in the decompression being done in stages but in its being rapid till the absolute pressure is halved and slow afterwards.

¹ Whether the law holds good for pressures much exceeding six atmospheres is still doubtful, as no experimental data exist.

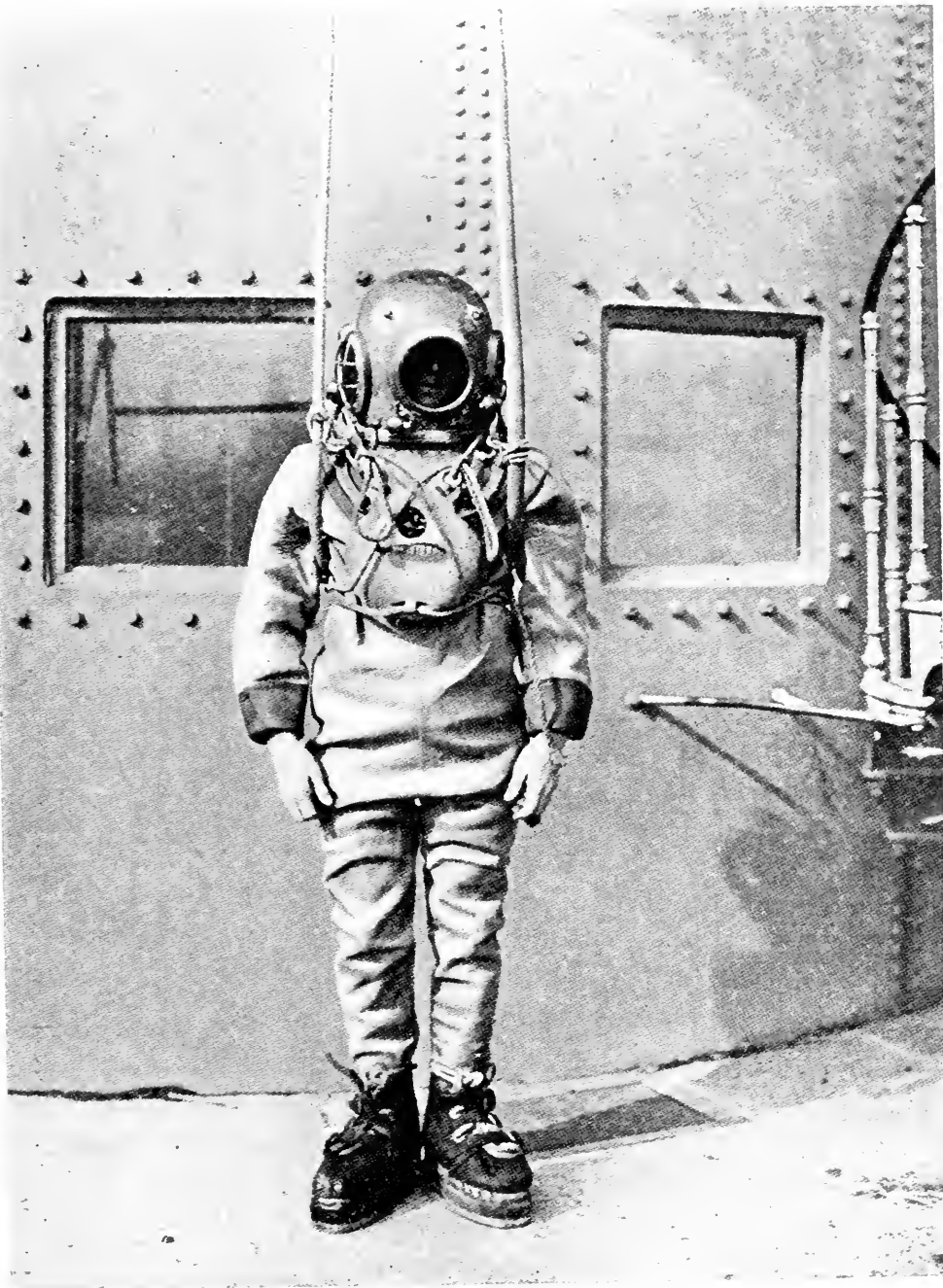
D. Practical measures for avoiding Compressed-air Illness.

From the foregoing discussion the general nature of the measures needed to prevent compressed-air illness will be evident enough. The risks may best be avoided by properly calculated stage decompression, or by cutting down the period of exposure to a safe limit, or by both methods combined. In the case of work in compressed air in caissons, tunnels, etc., it is for economic reasons very undesirable to greatly reduce the period of exposure. In diving work, on the other hand, the periods of exposure are generally short in any case, and they can, without great inconvenience, be confined within limits which largely reduce the risks of compressed-air illness. Long periods of decompression are also very undesirable in diving, since changes of weather or tide or other causes may render a return to the surface necessary without any long delay in coming up, and since very prolonged stays under water are exhausting, and the diver's hands may become benumbed by cold.

As our investigations were in the first instance made with the object of securing safety from compressed-air illness in diving work, we may first consider the precautions desirable in connection with diving.

(1) Diving work.

The ordinary diving dress (Plate IV) consists of a copper helmet screwed to a corselet, the latter being in its turn connected water-tight to a stout water-proof dress covering every part of the body except the hands, which project through elastic cuffs. Air is supplied through a non-return valve on the helmet from a flexible pipe connected with an air-pump on a boat or ship. The air escapes through an adjustable spring valve at the side of the helmet. The arrangement is thus such that the pressure of the helmet air breathed by the diver is always at least equal to, and usually slightly greater than, the pressure of the water at the valve outlet. At a depth of 33 feet or 10 metres the diver is therefore breathing air at an excess pressure of one atmosphere, or at an absolute pressure of two atmospheres; and every additional 33 feet will add another atmosphere to the pressure. To enable the diver to sink, the dress and boots are suitably weighted. He is usually in connection with surface by a life-line containing a telephone wire, as well as by the air-pipe.



Diving dress, front view, with air-pipe and life-line, which are connected with the helmet behind.

In descending or ascending a diver usually makes use of a rope attached to a heavy sinker at the bottom. He can thus easily regulate the rate of his ascent or descent, and take care that this rate is not so rapid as to cause any discomfort or pain in the ears owing to incomplete opening of the Eustachian tubes. A too rapid descent or ascent might cause mechanical injury followed by middle ear inflammation.

As explained above, there appears to be practically no risk of symptoms occurring from liberation of gas bubbles on rapid decompression if the pressure has not exceeded 1.25 atmospheres, corresponding to a depth of about seven fathoms or 42 feet of sea water. Up to this depth therefore no special precautions against caisson disease need be taken¹. At greater depths precautions depending on the duration of exposure are evidently needed. The precautions which we have calculated to be desirable are embodied in the table given below (Appendix IV.); and the principles and experimental results on which this table is based must now be discussed.

It will be convenient to consider first the case of diving to a very great depth, and we shall take as an extreme example the case of exposure at a depth of $35\frac{1}{2}$ fathoms (213 feet) of sea water, corresponding to an excess pressure of nearly 6.5 atmospheres, or an absolute pressure of 7.5 atmospheres.

Let us first suppose that the body of a diver is completely saturated with the nitrogen of air at this pressure, and that it is required to conduct his ascent to surface as rapidly as possible but without any risk of symptoms due to bubble formation, *i.e.* in such a way that, in accordance with the principles already laid down, the nitrogen pressure in no part of the body shall ever be more than double that of the air breathed at the same time.

The first step would obviously be to reduce the absolute pressure to about half, *i.e.* from 7.5 atmospheres absolute to 3.75 or from 6.5 atmospheres in excess to 2.75. This would be *ex hypothesi* the greatest initial drop in pressure which would be perfectly safe. The remainder of the decompression would evidently need to be conducted in such a way that the maximum partial pressure of nitrogen in any part of the body should diminish at double the rate of the fall in absolute pressure of the air. The ascent of a diver can be conveniently regulated from

¹ Heller, Mager and v. Schrötter recommend that at all depths decompression should be at a rate of at least 20 minutes per atmosphere. This would imply a delay of 25 minutes in coming up from 42 feet. Both common practical experience and our own experiments show that this excess of caution is quite unnecessary at small depths.

the surface by signalling to him to stop or come on at every ten feet as indicated on the pressure gauge attached to the pump. We may therefore divide the ascent into stages of ten feet, and the short periods occupied in the actual ascents may be neglected.

Since the depth was 213 feet, corresponding to 246 feet of water in absolute pressure, it would be safe to come up at once to a depth corresponding to 123 feet of absolute water pressure, *i.e.* to 90 feet of actual depth. Consequently the first stage would be a rapid ascent of 123 feet, and it would be necessary to wait here before the next ascent of 10 feet until the maximum partial pressure of nitrogen in the body had fallen to that of the nitrogen in air at $2 \times (80 + 33) = 226$ feet of absolute water pressure. The difference between 246 and 226 is 20, and this is 16% of $213 - 90 = 123$, the difference between the original and the reduced pressure. The most slowly desaturating parts of the body will, according to our previous calculations, take 75 minutes to give off half of any excess of nitrogen which they may contain at any given air pressure; by inspection of the curve (Fig. 1) it will be seen that they will take about 19 minutes to lose 16% of the excess. Hence a delay of 19 minutes would be necessary at 90 feet before coming up to 80 feet. At 80 feet the partial pressure in the body would require to fall an amount corresponding to 20 feet, which is about $17\frac{1}{2}\%$ of $193 - 80 = 113$, the new difference in relative pressure between the nitrogen in the body and in the air. This would necessitate a delay of 21 minutes before ascending to 70 feet. The further delays needed would be 23 minutes at 70 feet, 26 minutes at 60 feet, 30 minutes at 50 feet, 35 minutes at 40 feet, 42 minutes at 30 feet, 51 minutes at 20 feet, and 62 minutes at 10 feet. It would thus take 309 minutes, or more than five hours, to reach surface.

This calculation is represented graphically in Fig. 3. It will be noticed from the figure that the time required for safe decompression does not increase proportionally to the increase in depth. For instance, an increase in depth of 15 feet from 50 to 63 feet necessitates an increase of 45 minutes in the time required for safe decompression; but the same increase in depth from 198 to 213 feet only requires an increase of 15 minutes in the time of decompression.

A somewhat more rapid rate of stage decompression could probably be adopted without appreciable risk to life, but the occurrence under water of even one of the less serious decompression symptoms might be extremely unpleasant or indirectly dangerous, so that a factor which we believe to be thoroughly safe in this respect has been used in the

calculation. The possible occurrence of slight symptoms after surface had been reached would not, however, be a serious matter: for this reason half of the last stop at 10 feet from surface might be dispensed with, which would save half an hour. The most slowly desaturating tissues would, according to the calculation, still be only saturated to an excess pressure of 1.3 atmospheres—a safe enough limit perhaps, but leaving no great margin to spare.

Fig. 3 also shows the maximum excess of saturation with uniform decompression in the same time and in 10 hours. It will be seen that uniform decompression in about five hours would leave at the end of decompression an excess saturation within the body of 2.1 atmospheres; and even if uniform decompression were extended to ten hours the excess saturation would still exceed one atmosphere. It is also perfectly clear that uniform decompression is an unsuitable way of bringing a man out of compressed air. Where a sufficiently safe rate of uniform decompression is employed (as, for instance, with 10 hours in the case under consideration), it is only at the very end (when the nitrogen pressure inside the body becomes more than double that of the air) that there is any risk of symptoms occurring; and for the sake of safety at the end the whole process is made quite unnecessarily long. Increased safety at the end is only secured in combination with useless delay at the beginning¹.

As will be seen in Part II, the results of our experiments, allowance being made for the difference between goats and men, fully confirm the foregoing mode of calculation. Not only has stage decompression in the calculated time proved safe where uniform decompression in the same total time was unsafe, but shorter periods of stage decompression than those calculated have been proved to involve risk of symptoms, increasing in gravity and frequency with the shortening of the time, though always less than the risk from uniform decompression in the same time.

If the whole body of a diver were allowed to become saturated at any great depth, it is evident that the time needed for safe decompression would be impracticably long. To reduce the time of de-

¹ The regulations of the Dutch Government make the following method of decompression obligatory for work in caissons, &c. The pressure is to be lowered at the rate of not more than $\frac{1}{10}$ th of an atmosphere in 3 minutes till 3 atmospheres of excess pressure is reached: then at not more than $\frac{1}{10}$ th of an atmosphere in 2 minutes till $1\frac{1}{2}$ atmospheres excess pressure is reached; and finally at not more than $\frac{1}{10}$ th of an atmosphere in $1\frac{1}{2}$ minutes till normal pressure is reached. This method is still more unsuitable than uniform decompression, and would be very unsafe with high pressures.

compression to within limits practicable for divers, it is evidently necessary to greatly reduce the period of exposure to high pressure¹. At great depths limitation of the exposure is also necessary in order to avoid toxic effects from the high pressure of oxygen (see p. 371). Calculation of the mode and period of decompression required after a limited exposure to pressure is a somewhat complicated matter, but the principles already laid down render it quite possible.

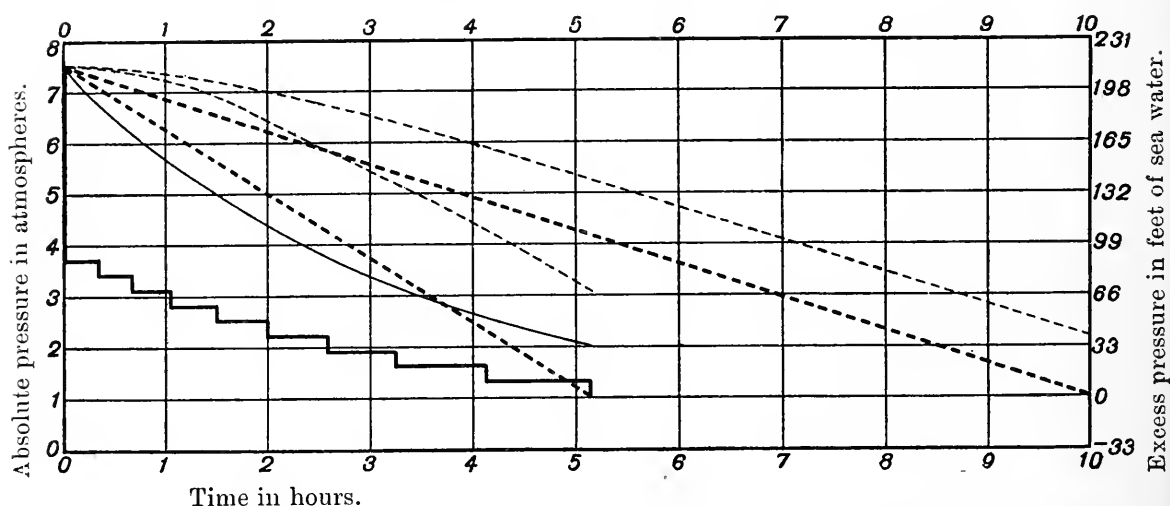


Fig. 3. Theoretical ascents of a diver after a prolonged stay at 213 feet of sea water. Stage decompression in 309 minutes compared with uniform decompressions in 309 minutes and in 10 hours. Continuous lines = stage decompression: interrupted lines = uniform decompression. Thick lines = air pressure: thin lines = saturation with atmospheric nitrogen in parts of the body which half saturate in 75 minutes.

When a diver goes down for a very short time, we have to take into consideration not only the time which he spends at the maximum pressure on the bottom but also the time occupied in the descent and the ascent. During the descent he is all the time saturating himself with nitrogen, and during most of the ascent he may be doing so also. Calculation will show that, if he descends and ascends at a uniform rate, the time spent in this process will be nearly equivalent, as regards the saturation of the body with nitrogen, to half the same time spent at the maximum depth. It is therefore clear that in deep diving the diver should descend as rapidly as is practicable, and should also ascend at once, on completion of his work, as far as he safely can. The rate of descent may be limited either by pain in the ears or by an air supply insufficient to keep the upper part of the dress full of air.

¹ This was fully realised by Catsaras who recommended a stay on the bottom of only 1 minute at 30 fathoms.

Both these causes are avoidable, and an experienced diver, with his Eustachian tubes well opened and a proper supply of air, can get to an excess pressure of six atmospheres (198 feet) in two minutes. This time was found sufficient in experimental dives up to 210 feet made by Lieut. Damant and Mr Catto (Appendix II). The recommendation commonly made that the rate of both ascent and descent should be slow is evidently quite unsound. A man who spent half an hour in descending to 30 fathoms, and an equal time in ascending at a uniform rate, would run a considerable risk of perishing on his return to the surface.

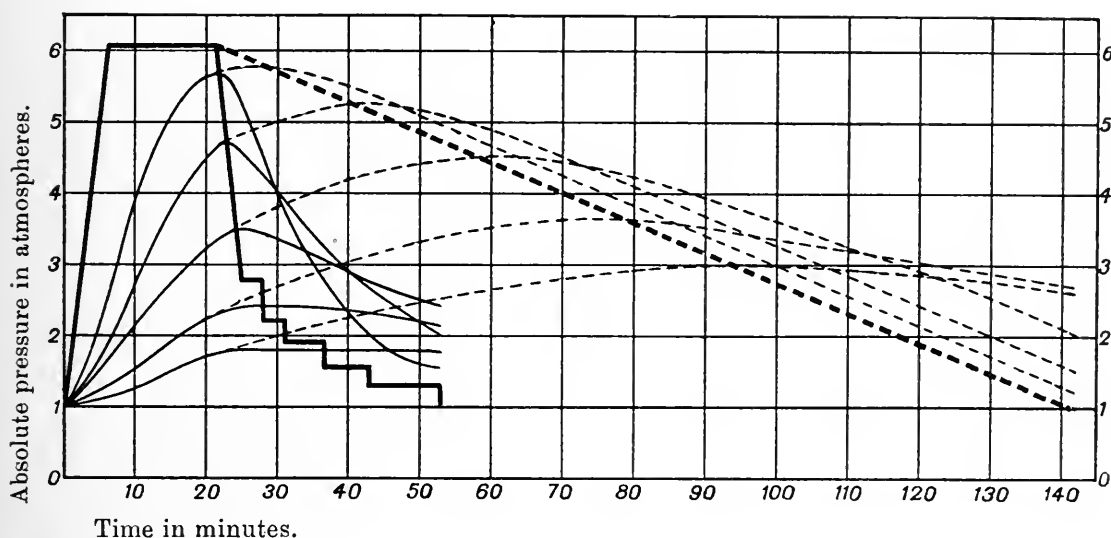


Fig. 4. Desaturation during stage decompression in 32 minutes and uniform decompression in 2 hours, after exposure for 15 minutes at 75 lbs. pressure with compression in 6 minutes. Thick lines=air pressure: continuous lines=stage decompression: dotted lines=uniform decompression. The curves from above downwards represent respectively the variations in saturation with nitrogen of parts of the body which half saturate in 5, 10, 20, 40, and 75 minutes.

In order to illustrate the method by which we have calculated safe modes of ascent in the minimum period of time we may take as an example the case of exposure for 15 minutes to a pressure of 75 pounds (6.1 atmospheres absolute or 28 fathoms = 168 feet). Many of our experiments on goats were made with this pressure and exposure. It took about six minutes to raise the pressure in the experimental chamber to 75 pounds, so that the total virtual exposure till decompression began was about 18 minutes. Fig. 4 shows graphically the variations of pressure during this period: also the calculated partial pressure of nitrogen in different parts of the body, as compared with the nitrogen pressure in the air. The first stage was from 6.1 to 2.8

atmospheres absolute (corresponding to an ascent in sea water from 168 to 60 feet) and occupied four minutes. The subsequent stoppages were:—

2	minutes	at	2·8	atmospheres	(60	feet	of	sea	water),
3	„		2·2	„	(40	„),
5	„		1·9	„	(30	„),
7	„		1·6	„	(20	„),
10	„		1·3	„	(10	„).

It will be seen from the figure that this rate of decompression was slightly faster than what was calculated above to be desirable. At the end of decompression the nitrogen pressure in those parts of the body which became half saturated in about 20 minutes under pressure would be equivalent to that of air at about 1·4 atmospheres, or 20·6 pounds per square inch. If the circulation in one of these parts were less vigorous during decompression than during exposure to the high pressure, it might well be that the nitrogen pressure in this part at the end of decompression would be higher than corresponded to the calculation. As a matter of fact minor symptoms (“bends”) were observed five times in 34 decompressions of 18 goats, although no serious effects occurred. We concluded that the period of virtual exposure (18 minutes) was slightly longer than is desirable with stage decompression in 31 minutes: in the table below (p. 442) the limit has been set down at 15 minutes.

Fig. 5 shows the calculated nitrogen pressure in different parts of the body during uniform decompression in 31 minutes after the same exposure at 75 pounds. It will be noticed that at the end of decompression there is a dangerous excess of saturation in all parts of the body except those which half saturate in less than about seven or eight minutes, and that this supersaturation corresponds to an excess pressure of as much as 2·1 atmospheres of air. The goats used for the stage decompression experiments were on alternate occasions subjected to uniform decompression in the same time and with the same exposure. The result was that, in 36 decompressions, one died, two were paralysed, one had indefinite general symptoms of a severe character, and in 11 other cases “bends” occurred, besides two doubtful cases. This was entirely in accord with what the calculation would lead us to expect; and uniform decompression in 31 minutes is evidently dangerous under the conditions given.

It might be supposed that safety would be secured by extending to

a moderate degree the length of uniform decompression. It must be remembered however that the more the duration of uniform decompression is extended, the longer is the period during which the body is exposed to high pressure. Fig. 4 shows the calculated effects of uniform decompression extended to two hours. Although the quickly saturating parts of the body are desaturating during the greater part of the decompression, the slowly saturating parts are, on the other hand, becoming more and more saturated, so that at the end of decompression the parts which half saturate in from 40 to 75 minutes are saturated to an excess pressure of about 1·7 atmospheres, although at the beginning of decompression they were only saturated to from 0·7 to 1·3 atmospheres and could consequently have given no trouble.

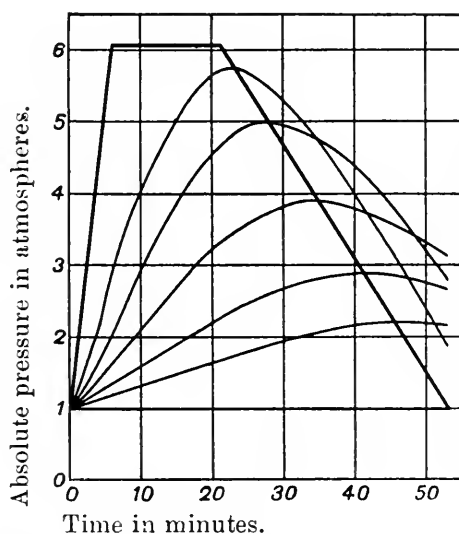


Fig. 5. Desaturation during uniform decompression in 32 minutes after exposure for 15 minutes at 75 lbs. pressure with compression in 6 minutes. Thick line=air pressure. The curves from above downwards represent respectively the variations in saturation with nitrogen of parts of the body which half saturate in 5, 10, 20, 40 and 75 minutes.

Very prolonged uniform decompressions are extremely tedious, and it seemed scarcely worth while to make any extensive series of such experiments. We found however that out of 12 goats uniformly decompressed in 90 minutes after 18 minutes virtual exposure at 75 pounds (6·1 atmospheres of absolute pressure) three developed symptoms of bends after decompression. The proportion of illnesses was thus greater than with stage decompression in a third of the time. With men the results would certainly be much worse, and we calculate that for a man, after the same exposure, several hours would be needed for uniform decompression in order to escape all risk of

symptoms occurring. The time would in fact require to be nearly as long as if the body had been completely saturated at the maximum pressure.

With very short exposures to high pressure, rapid decompression is probably safer than uniform decompression at a moderate rate. There is a considerable human experience on this point: divers working at great depths would seem to consider it fairly safe to go rapidly to the bottom at a depth of 160 or 180 feet and return equally rapidly, provided the time spent on the bottom does not exceed six or eight minutes and provided also that the dives are not repeated at short intervals. It is reported of the skilled Greek divers of the Mediterranean that, in case their gear becomes entangled on the bottom, they will cut their air-pipe and line and blow themselves up to the surface in less than a minute from a depth of 30 fathoms or the like rather than stop more than about ten minutes on the bottom. Our experiments on goats are in accordance with this practice. We found that no symptoms were produced by sudden decompression in less than a minute after virtual exposures at 75 pounds up to four minutes, and even in some trials up to six minutes (see below, p. 394).

With exposures exceeding a very few minutes, or such brief exposures frequently repeated, so that during the intervals the body has not time to become desaturated, we have little doubt that slow and uniform decompression—the slower the better—is at any rate preferable to sudden decompression. Uniform decompression must however be extremely slow to make it entirely free from risk of death or very serious symptoms, and the time required is so great that this method seems to us quite impracticable in connection with diving work. There appears to be very little human experience of slow uniform decompression. Divers usually come up in a few minutes at most, and even half an hour spent in the ascent would appear to be quite exceptional. Almost the only definite observations are those of Hill and Greenwood, who recently experimented on themselves at very high pressures. Fig. 6 shows the variations of pressure and the calculated saturations of different parts of the body during the experiment in which Greenwood went to a pressure of 91 pounds (7.1 atmospheres absolute). This experiment appears to have been a very risky one. After decompression he had bends in both arms, and Hill also had symptoms pointing towards blockage of vessels in the subcutaneous fat after similar experiences at 75 pounds pressure.

In Appendix IV two tables are given for the safe decompression of

divers after exposure for varying periods of time at different depths. These tables are the same as are now in use for divers in the Royal Navy, on the recommendation of the Committee on Deep Diving. In Table I the period of virtual exposure is so limited that the diver can return to surface by stages in half an hour or less. It will be noted that the maximum periods of exposure are from the time of leaving surface, so that there should be no chance of increased danger from undue delay in descending. The stoppages during the ascent are so calculated that, until surface is nearly reached, the excess of nitrogen pressure in any part of the body should never be more than double the nitrogen pressure of the air breathed, and not more

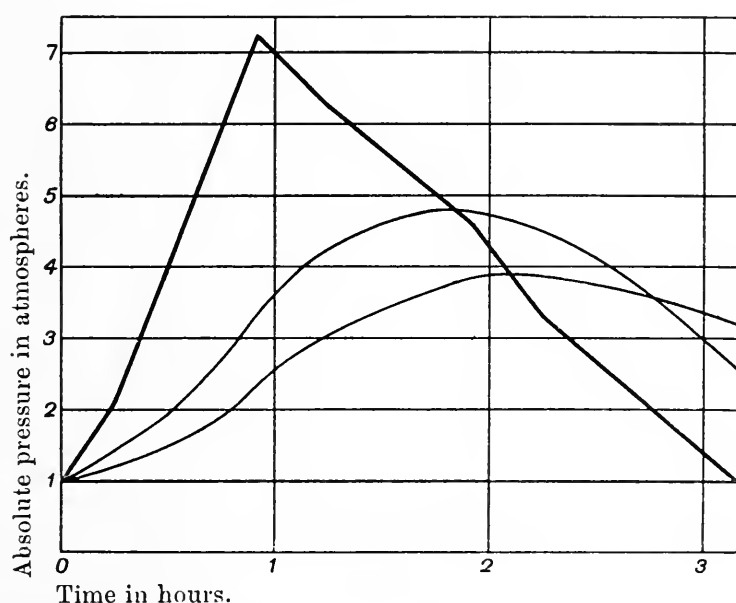


Fig. 6. Showing calculated variations in saturation with nitrogen during Dr Greenwood's experiment on himself. Thick line=air pressure: the two curves from above downwards represent respectively the variations in saturation with nitrogen of parts of the body which half-saturate in 40 and 75 minutes.

than two and a quarter times this pressure when surface is reached. The only case in which these limits are allowed to be slightly exceeded is with short exposures in comparatively shallow water. This slight excess is, however, only in parts of the body which saturate and desaturate very rapidly, and, as already explained, give rise to no danger. As an additional safeguard the diver is directed to keep his arms and legs constantly moving during each stoppage, so as to increase the rate of circulation and guard against the chance of the rate of desaturation during his ascent being proportionally less than the rate of saturation during his stay on the bottom while he was doing work.

The second table provides for the case of exceptionally long stays under water. A diver may be delayed by his air-pipe or life-line being fouled, or by other exceptional circumstances, against which it is necessary to provide. Where the fouling has been complicated by the action of tide the delay on the bottom has occasionally amounted to several hours, until the tide has slackened or turned. If the diver is at a great depth the calculated time required for safe decompression after so prolonged a stay is very long. On the other hand the dangers from cold and exhaustion have to be considered, and the difficulties caused by a strong tide during the diver's ascent. In view of these difficulties the time allowed for decompression after very prolonged exposures is somewhat curtailed, but not so much as to permit of risk of more serious symptoms than "bends," in so far as experiments on animals, and human experience, render it possible to calculate. In the case of men of exceptionally heavy build, and inclined to obesity, the time allowed after very prolonged exposures ought to be increased by about a third, although such men, particularly if over about 45 years of age, ought not to expose themselves to the risk of a prolonged stay in very deep water.

It might appear as if the rate of stage decompression recommended after prolonged exposures was slower than is actually required. A very unfortunate accident which occurred recently has shown only too clearly that this is not the case. In connection with the work of raising a torpedo boat which had sunk in 25 fathoms (150 feet or 46 metres) several divers were employed. They were working in 20 minute spells, and returning to surface by stages in 32 minutes, in accordance with the first table, which was the only one then in use. No symptoms of any kind were observed after the divers' return to surface under these conditions, nor have any symptoms ever been observed hitherto among divers working according to the table. One of the divers, however, became fouled in a very exceptional manner. His life-line was fixed in one direction over a spar or rope belonging to the sunk vessel, and his air-pipe was fixed in the other direction. He was thus prevented from going to free either his air-pipe or life-line. A second diver at once went down, but was unable to free him owing to the drag caused by the tide; and it was only after two and a half hours, when the tide had slackened, that he got free. He was then brought up by stages under the direction of Staff Surgeon Rees and Lieut. Damant. For the decompression two and a half hours were allowed, which we then believed would be a sufficient time in case of a diver being badly fouled

at 25 fathoms. The diver was, however, a man of heavy build with much fat in his body, and aged 49. Owing to his exhausted condition he did not come up on the ordinary rope, but had to be pulled up, hanging motionless on the life-line during the long stoppages. On reaching surface he was very exhausted and could hardly have been safely kept longer in the water. He had no paralysis or other definite symptoms of caisson disease. A bed was arranged for him on deck, and hot bottles &c. applied. After a time he complained of some pain in the legs, but this soon subsided; and as he seemed much better in the morning he was removed to hospital, since there was no suitable accommodation for him on the gun-boat where he was. The moving made him worse, and he gradually became restless and delirious in spite of administration of oxygen at intervals, showed signs of cardiac failure, and died somewhat suddenly about 24 hours after he had been brought up. At the post-mortem examination 12 hours later a moderate number of bubbles were found in the right side of the heart, the veins of the liver and intestines, while scattered bubbles were present in vessels elsewhere, including the coronary vessels, though none were seen in the vessels of the brain. The mesenteric fat, which was very abundant, was in places distended with small bubbles. There was about an inch of subcutaneous fat over the trunk, but no bubbles were seen in this layer. There seemed no reason to doubt that death was largely due to the bubbles, although the more usual symptoms of caisson disease were absent. There were no signs of pneumonia.

This is the only known case of prolonged exposure of a man to such a high excess pressure as four and a half atmospheres; and although his age, heavy build, and exhausted condition combined to make the circumstances very unfavourable, the fact of his death shows that the long decompression periods recommended in the second table after prolonged exposures are none too long, even for a man of ordinary build. Every precaution should be taken to guard against such long exposures at high pressures.

A diver has often to descend twice or oftener at short intervals. At the beginning of the second descent the more slowly desaturating parts of the body will not have had time to lose their excess of nitrogen, and consequently they will be more highly saturated at the end of the second descent than would otherwise have been the case. This will be clear from a study of Figs. 2 or 3. To meet the increased risk in decompression it is desirable, in calculating the proper stoppages, to add together the two periods of exposure, and adopt the corresponding rate

of decompression shown in the tables. For the first half of the stoppages this is not necessary, but for the second half, including the longer stoppages needed to meet the case of the more slowly desaturating parts, the rule should be carried out. The increasing danger after successive short dives by pearl divers, &c. without any precautions in decompression is notorious. This danger does not mount up to the same extent with stage decompression, but nevertheless exists. As the interval between successive dives increases the added danger on decompression diminishes. With an hour's interval the extra precautions might be halved, and with two or three hours' interval they might be omitted.

It may be remarked that the precautions recommended in the tables are greatly in excess of those which have hitherto been commonly employed in either diving work, or work in caissons, tunnels, &c. We have endeavoured to leave a clear margin beyond everything which either human experience or experiments on animals, or calculation, has shown to be risky. In connection with diving, the practice hitherto recommended in the British and other navies has been that the diver should both descend and ascend at a uniform slow rate. By abolishing the slow descent and ascent, and substituting stage decompression, it has been possible to combine greater safety with a clear saving of time under water for a given working period on the bottom. Where the air supply to the diver is managed in accordance with the recommendations of the Diving Committee there is also very greatly increased working efficiency in deep water. For a discussion of the air supply to divers, and many other practical points relating to diving, we must refer to the Committee's Report; and to the "Diving Manual," which has just been re-written and issued to the Royal Navy.

A possible complication to which we have not hitherto referred in connection with compressed-air illness arises from the fact that at very high pressures of air the partial pressure of oxygen begins to be so high as to be capable of producing serious effects. Paul Bert discovered that oxygen at a partial pressure exceeding about three atmospheres (corresponding to 14·3 atmospheres of air) causes animals to go into convulsions and die, even a short exposure being often fatal. More recently, Lorrain Smith, who experimented on mice, and whose results have been confirmed and extended by Hill and Macleod, showed that oxygen at high pressure acts on the lungs, producing pneumonia¹. He

¹ Lorrain Smith, *Journ. of Physiology*, vol. xxiv., p. 19, 1899; Hill and Macleod, *Journ. of Hygiene*, vol. iii., p. 401, 1903.

found that fatal pneumonia may be produced after four days' exposure to an oxygen pressure of as little as 75 % of an atmosphere, corresponding to air at an absolute pressure of 3·6 atmospheres (88 feet of sea water). At a pressure of about 1·25 atmospheres of oxygen (6 atmospheres of air, or 165 feet of water) death from pneumonia was produced in about 48 hours. At about 1·8 atmospheres of oxygen (eight and a half atmospheres of air, or 250 feet of water), marked symptoms usually occurred in about 12 hours, and death in 20 hours, though in one case death followed in seven hours. At about 2·8 atmospheres of oxygen (13·3 atmospheres of air, or 406 feet of water) marked symptoms were observed in about three hours, and death in nine hours.

The steel chamber at the Lister Institute was not made to withstand such high pressures as would produce within a short time symptoms of oxygen poisoning if air alone was pumped into the chamber. We have, however, made a few observations in the chamber when the oxygen pressure of the air breathed was raised by other means. In one experiment seven goats were placed in the chamber, and the oxygen pressure raised by opening three large cylinders of oxygen, and at the same time pumping in air to 81 pounds pressure. The total oxygen pressure was thus raised to 2·3 atmospheres, corresponding to a depth of 55 fathoms, or 330 feet, or 100 metres. After three hours one animal had died of pneumonia in the chamber, and most of the others seemed more or less affected, though they rapidly recovered on decompression¹. We also tried on ourselves the effects of breathing nearly pure oxygen from a bag while we were in the chamber at an absolute pressure of two atmospheres; but we could not detect any effects after a few minutes with an oxygen pressure of 1·7 atmospheres, corresponding to about 40 fathoms (240 feet or 73 metres). In a number of goats which were exposed to 75 pounds' pressure (168 feet or 51 metres of water) for three hours, no symptoms indicative of oxygen poisoning were observed.

To judge from these data there is no immediate risk to a diver from oxygen poisoning at depths up to 40, or perhaps 50 fathoms (73 to 90 metres) if ordinary air is breathed, provided the stay is not long. With stage decompression the diver could rapidly return to a perfectly safe oxygen pressure; but, as already remarked, we do not yet know with

¹ One animal showed bends after decompression which was effected in 133 minutes by stages. After exposure at +75 lbs. for 3 hours in air this decompression gave 2 bends in 14 goats. There is therefore no evidence that the exposure to high pressure oxygen increased the susceptibility to caisson disease.

certainty whether it is perfectly safe to rapidly reduce the pressure to half after exposure to such very high air pressures.

(2) *Work in caissons, tunnels, and diving bells.*

In connection with various kinds of engineering work under water, or in soft water-bearing strata, compressed air is commonly used for keeping water out of the working place and preventing collapses. The men have thus to work continuously in compressed air.

In tunnels or 'tubes' through soft water-bearing strata, where a steel lining has to be erected to keep water out and resist pressure, the working face, or blind end of the tunnel under construction, is kept free of water by the air pressure with the help of a circular shield with a cutting edge which is advanced as each section of steel lining is erected into position. The soil is excavated by hand labour, and passed out on trucks through an air-lock.

In constructing foundations for the piers of bridges over rivers, caissons are employed. A caisson is a steel tube, which ultimately forms the lining of the pier, and is shaped accordingly. Near the lower end there is a steel diaphragm, forming a working chamber. An inner steel tube passes through this diaphragm, and serves for ingress and egress, and for passing up the material excavated. At the top of this inner tube there are air-locks for allowing the passage of men and material without escape of the compressed air contained in the working chamber. The latter is kept free from water by the air pressure, and the excess of air escapes beneath the cutting edge of the caisson. When a secure foundation for the pier has been reached this chamber is filled up with concrete. In constructing mine shafts through soft water-bearing strata the same principle may be employed. For work of a simpler kind on river or harbour bottoms diving bells are often used, the bell being simply lowered to the bottom at any required place, so that the men can work on the area covered by it and are kept dry by the air pressure.

The circumstances connected with work in compressed air in caissons, tunnels, &c., differ in certain respects from those associated with diving work.

In the first place the duration of exposure is far longer. A caisson or tunnel worker is usually in compressed air for six or eight hours daily, or even longer. The conditions of the work render any great limitation of the periods of exposure very difficult and expensive.

Usually, however, the workman comes out for meals at intervals of about three hours.

A second difference is that the very high pressures to which a diver may have to go are not needed in caisson or tunnel work. An excess pressure of about $3\frac{1}{4}$ atmospheres, or 48 lbs., is, we believe, the extreme limit hitherto employed; and usually the excess pressure does not exceed about two atmospheres or 30 lbs. Decompression seems to be usually effected in 10 to 20 minutes, or even, with the lower pressures, in three to five minutes.

With properly arranged air-locks for men and material there should be no need for hurry in coming out; and undue hurry is specially undesirable if the workman leaves the works at once, since he would be liable to develop symptoms when he was so far away that he could not be readily recompressed. To obviate this risk as far as possible, it is customary to endeavour to keep men for half to one hour on the works after they come out; and with the usual rates of uniform decompression this precaution is very necessary. Evidently, however, it is greatly preferable to prevent all practical risks of serious symptoms.

In order to attain this end stage decompression as recommended for divers in the tables in Appendix IV may be employed. An accurate and easily read pressure gauge, visible from both inside and outside the air-lock, is of course essential; and a reliable man should be in charge of the tap. As a further control it would be desirable to have an automatic graphic record of the variations of pressure each time the lock for men is used. As any very sudden drop in pressure might cause mechanical injury, the outlet tap should be so arranged as to prevent decompression at a maximum initial rate of more than about one pound in five seconds¹. With this arrangement and an ordinary tap, the rate of decompression would diminish considerably as the pressure fell, and the proper point for interrupting the decompression could be accurately reached.

The tables in Appendix IV have been calculated with special regard to the comparatively short periods of exposure to pressure in diving work;

¹ The delivery of the inlet tap should also be restricted, and the man in charge should have strict directions to take care that the rate of admission or discharge of air does not cause pain in the ears, &c. of any of the men in the lock. To avoid pain a very slow rate of air admission may sometimes be needed, but with practice a rise of pressure of one atmosphere per minute is often not too much, so that any definite rule, limiting the rate to much less than this, seems scarcely desirable.

and the stoppages recommended during the divers' ascent after exceptionally long periods of exposure are somewhat shorter than would be desirable apart from the risks entailed by the long stay under water. In the case of caisson and tunnel workers, on the other hand, it is only in exceptional cases that the exposure to pressure lasts less than three hours; and usually the exposure during the day lasts at least six hours.

With such long exposures and only moderate pressures the calculated theoretical rate of safe decompression after the first rapid stage is nearly uniform; and the rules for decompression may be greatly simplified by adopting uniform slow decompression or uniform stages¹.

The following table shows the rate of uniform slow decompression calculated to be safe after the initial diminution of absolute pressure in the proportion of 2 : 1. Suppose, for instance, that men were working at a pressure of 24 pounds in 3-hour spells, with an hour's interval between for a meal. In coming out they would be rapidly decompressed to an absolute pressure of $\frac{24 + 15}{2} = 19\frac{1}{2}$ pounds or 4½ pounds of excess pressure. After the first 3-hour spell of work the slow decompression would be at the rate of one pound in three minutes, or $3 \times 4\frac{1}{2} = 13\frac{1}{2}$ minutes in all. After the second spell the rate would be one pound in five minutes, corresponding to $22\frac{1}{2}$ minutes in all. If they stayed for the whole period in the compressed air the rate of slow decompression would be one pound in seven minutes corresponding to $31\frac{1}{2}$ minutes in all. To take another example, if the work were at 40 pounds excess pressure the men could be rapidly decompressed to $\frac{40 + 15}{2} = 27\frac{1}{2}$ pounds of absolute pressure, or $12\frac{1}{2}$ pounds excess pressure. After a first 3-hour spell of work the period of slow decompression would therefore be $12\frac{1}{2} \times 7 = 87$ minutes: after a second spell (with an interval of 30 or 45 minutes outside the lock) $12\frac{1}{2} \times 8 = 100$ minutes; and after a continuous exposure of six or seven hours, $12\frac{1}{2} \times 9 = 112$ minutes².

¹ With the lock air-tight, and no ventilation, uniform decompression at any required rate could be easily secured by means of a reducing valve on an outlet, with a graduated tap beyond it, the arrangement being similar to the reducing valve and tap usually connected to a cylinder of compressed oxygen or gas used for limelight. If the delay in the lock is so long that ventilation is required, or if ventilation is needed in order to compensate for accidental leakage, it would be best to have an adjustable safety valve on the outlet, and adjust this by one pound at a time at the proper intervals.

² We have some doubt as to whether the increased slowness of decompression after very long exposures would be altogether sufficient to meet the increased tendency to slight symptoms ("bends"). These are, however, of minor importance if all serious symptoms

TABLE I.

Table showing rate of decompression in caisson and tunnel work.

Working pressure in pounds per square inch	Number of minutes for each pound of decompression after the first rapid stage		
	After first three hours' exposure	After second or third three hours' exposure, following an interval for a meal	After six hours or more of continuous exposure
18—20 pounds	2	3	5
21—24 „	3	5	7
25—29 „	5	7	8
30—34 „	6	7	9
35—39 „	7	8	9
40—45 „	7	8	9

It will be evident from the last example that in order to avoid waste of time in the lock it would be preferable with pressures exceeding about 25 pounds to keep the men under pressure continuously during each shift. Thus with two 3-hour spells of work separated by a decompression, the time spent in the lock would be $87 + 100 = 187$ minutes; whereas if the meal were taken in the compressed air, the two 3-hour spells would only imply 112 minutes in the lock.

With working pressures exceeding about 25 pounds the air-lock should be roomy and comfortably arranged, and large enough to take the whole of a shift of men. It should be provided with an electric heater, telephone, and if possible some sort of lavatory accommodation.

With pressures up to 45 pounds, or four atmospheres of absolute pressure, there appears to be no substantial objection to keeping men for six hours, or even more, continuously under pressure, provided that the mode of decompression is thoroughly safe. With pressures exceeding about 40 pounds, the practice has hitherto been to limit the exposure to about one hour, and employ rates of decompression which are dangerously rapid. This plan implies greatly increased risk and expense, since for the accomplishment of the work the number of decompressions is six times as great, and the men are idle most of the day. The actual increase in risk must be very great.

In tunnel work, or any other kind of work where plenty of space is available, there would be great advantage in providing a large air-lock, or section of tunnel, in which the pressure was constantly maintained at a little less than half the absolute pressure in the working section.

are prevented. We also think that with long shifts, exceeding a total of about 3 hours, still slower decompression would be needed for any men inclined to obesity. Such men should, therefore, be excluded in the medical examination which all men working in air at high pressures ought previously to undergo.

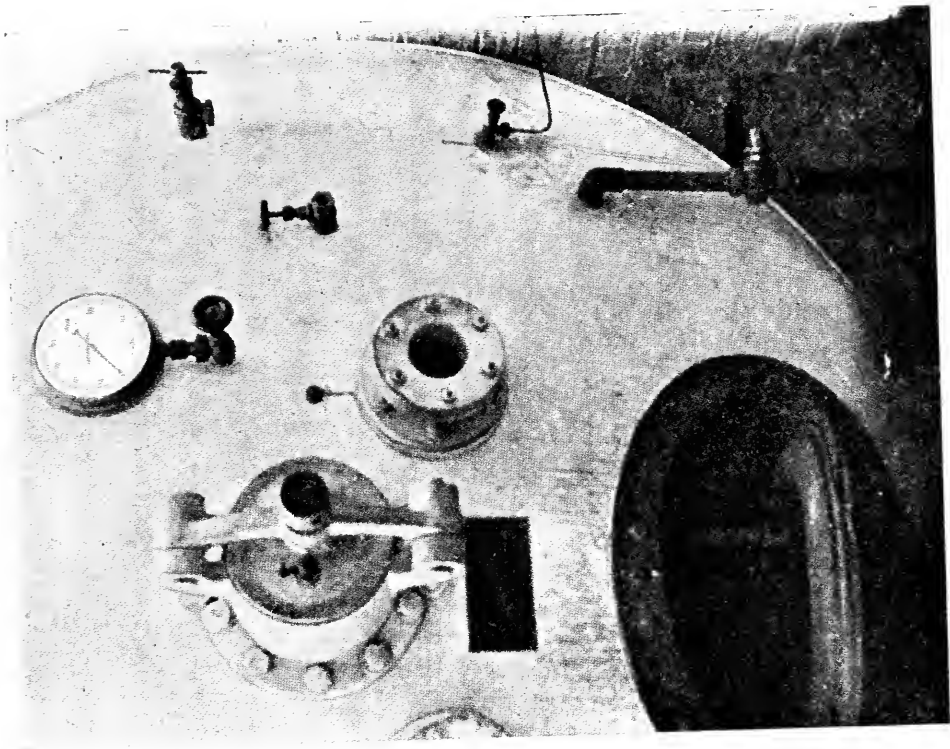
The men could then pass rapidly (in two or three minutes) from the working section into this intermediate lock or section, where they could take their meals, wash, and change their clothes. After a sufficient delay (dependent on the working pressure) they could then pass out rapidly. If, for instance, the working section was at a pressure of 30 pounds, the intermediate or "purgatory" lock could be kept at an absolute pressure of about $\frac{30 + 15}{2.2} = 20.5$ lbs., or $5\frac{1}{2}$ pounds of excess pressure¹. At the end of the day's work there would be a delay of about 50 minutes in this large lock, during which the men could wash and change, or take a meal. With this plan all delays during actual decompression would be obviated, so that ingress and egress would be free at all times, and the men could use the locks employed for material. For persons going in for only short periods the delay in the "purgatory" lock could be curtailed in accordance with the tables in Appendix IV. The movement of the men while employed in washing, changing clothes, &c. would hasten the process of desaturation, and this would be a further advantage.

In any case where it was specially desirable to reduce the period of delay in the air-lock to a minimum, recourse could of course be had to breathing oxygen during the period of slow decompression. This would about double the rate of desaturation, and therefore halve the delay. The oxygen could be breathed from a bag, and the CO₂ absorbed by a purifier, so that very little oxygen would be needed. By so arranging the mouthpiece that part of the expired CO₂ was rebreathed, and the respiration and circulation thus stimulated, a still better result would be attained.

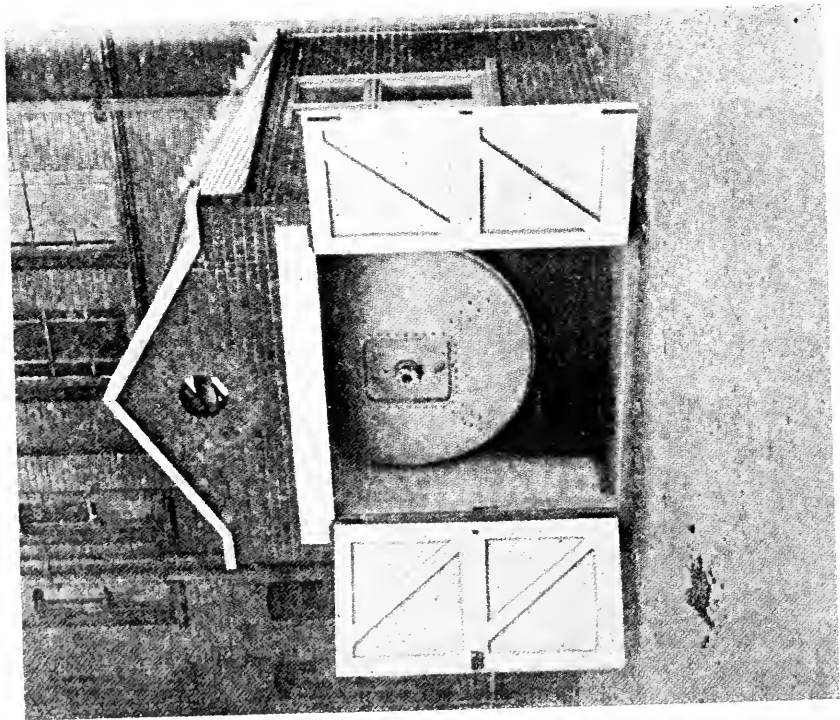
The results of some of our experiments seem to indicate that even the very slow rate of stage decompression which has been recommended above would be insufficient to completely obviate the risk of "bends" occurring after prolonged exposure. The rate of saturation and desaturation of some of the tissues which are the seat of "bends" is possibly slower than we have provisionally assumed. What we have aimed at is to completely obviate the risk of any serious symptom, while at the same time reducing the chances of "bends" to a minimum.

¹ A comparatively rapid fall in absolute pressure in the proportion of 2.2 to 1 is within practically safe limits, particularly if the previous period of continued exposure has not exceeded three or four hours.

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The steel chamber at the Lister Institute. Front end, showing the manhole for entering, the small air-lock for passing food, &c. into the chamber, an inspection window, a pressure gauge, and several valves, &c.



The steel chamber at the Lister Institute. View from outside, showing the back end of the chamber, with the large door and one inspection window.

PART II. EXPERIMENTAL.

1. *Apparatus.*

We owe the large pressure chamber (Plate V) in which both human and animal experiments were conducted to the generosity of Dr Ludwig Mond, F.R.S. It is a short segment of a boiler of $\frac{5}{8}$ inch plate resting on its side; the ends are slightly dished steel plates $\frac{1}{2}$ inch thick. Inside it measures $7\frac{1}{2}$ feet long by 7 feet wide and high, and has a capacity of 9500 litres (336 cubic feet). It is thus large enough to hold 3 or 4 persons comfortable and can be used for animal experiments lasting several hours without the necessity of ventilating. There are two doors: one, an oval manhole (24×15 inches), is easily removed and is in common use; at the other end is a large rectangular plate (28×24 inches) which can be unbolted for the admission of bulky articles. There are a number of spring and simple valves; the largest is in the floor of the chamber and serves also as a drain; when fully opened it reduces the pressure from 100 lbs. to atmospheric pressure in rather less than a minute. Besides this there are four spring and three simple valves so arranged that the pressure can be completely controlled either from inside or outside. The front is also furnished with an air-lock, by means of which small articles can be passed in or out of the chamber during an experiment. Three windows are provided of stout glass; as a precaution for safety these are fitted with an arrangement whereby the breaking of the glass releases a solid metal rubber-faced plug which falls into the hole. Wiring for lights, a telephone, electric heaters and a motor to drive a fan, kymograph, &c., is introduced through fibre plugs.

The pressure is raised or reduced by a simple compressor driven by a gas engine. While this has proved quite satisfactory for negative pressure experiments, the rate at which the pressure can be raised by its means is only about 2 lbs. per minute. This was a serious obstacle to the examination of the effects of exposure to high pressures of short duration. Accordingly after the preliminary experiments, a multitubular compressed air reservoir was placed at our disposal by the Admiralty. This reservoir has a capacity of about 22 cubic feet, and by charging it to about 70 atmospheres with a two-stage liquid-air compressor and also another steel bottle to 180 atmospheres we were enabled to suddenly blow the contents into the chamber and so reach a pressure of 60 lbs. in

4 minutes, and 75 lbs. in $5\frac{1}{2}$ — $6\frac{1}{2}$ minutes according to the temperature. The pressure is indicated by two Schaffer spring gauges (one of which is visible from within the chamber) for positive pressures, and one spring gauge outside and a mercurial barometer inside for negative pressures. The spring gauges show a lag of nearly 2 lbs. up to about half an atmosphere, but above one atmosphere they are concordant and, as far as could be ascertained, correct.

The chamber and accessory apparatus have now been frequently used during eighteen months for experiments at pressures varying from 100 lbs. above to 8 lbs. below atmospheric pressure, and have been found very satisfactory and convenient.

2. *Choice of experimental animals.*

A few experiments were made with rabbits, guinea-pigs, rats, and mice, but for regular use goats were selected chiefly because they were the largest animals which could be conveniently dealt with and which could be obtained in considerable numbers. The questions under consideration depend in a very fundamental way upon the rate of circulation in the animal under investigation. Among the ordinary mammals this must vary with the rate of the respiratory exchange per unit of body weight and is therefore proportional to the ratio between body surface and body weight. The susceptibility of any animal to caisson disease after sufficiently long exposure to compressed air must depend in the main upon the rate at which its respiration and circulation removes the excess of dissolved nitrogen on decompression.

Not only is this excess removed more rapidly in small animals, so that the time during which bubbles might be formed is correspondingly less, but, as already pointed out, there is every reason to believe that the time during which the venous blood remains in a supersaturated state during each round of the circulation largely determines the formation of bubbles. This time is so short in small animals that no bubbles at all are formed, in spite of the temporary existence of very great supersaturation in the blood and tissues. The susceptibility of any species of animal then varies enormously with the size. Thus a mouse, weighing 20 grammes and with a CO_2 production of about 8 grammes per kilo per hour, is much less susceptible than a goat, weighing 20,000 grammes and producing about 0.8 gramme CO_2 . We have indeed failed to produce any symptoms at all in mice on decompression in less than a minute after one hour's exposure at 75 lbs., an experience

invariably fatal to goats. In the same way dogs, with a respiratory exchange of some 1.3 gms. CO₂ per kilo per hour, are much less susceptible than men with an exchange of about 0.5 gms. Thus Heller, Mager and v. Schrötter observed no symptoms in dogs¹ on sudden decompression from any pressure less than about 60 lbs., while abundant illnesses are caused in man, and for the matter of that in goats also, by inappropriate release from 30 lbs. pressure. It therefore appears clear that it is necessary to use large animals for experiments which are designed to illustrate the incidence of caisson disease in man. Indeed the quantitative factor by which the results obtained on quite small animals might be translated into human experience is so large as to become qualitative in character.

Since pressures of some 100 lbs. or more are required to produce symptoms in a reasonable proportion of small animals, the use of animals such as goats is also very desirable in order to keep as far away as possible from the point at which the partial pressure of oxygen is high enough to cause toxic effects. We found that an exposure of three hours at 81 lbs. to an atmosphere containing 36 % oxygen (the oxygen pressure being thus equal to that of 150 lbs. excess air pressure) killed one goat out of seven with "pneumonia." Our experience shows that it is not necessary to exceed an air pressure of half this (75 lbs.) to produce symptoms which are sufficiently varied and severe to satisfy experimental requirements.

Experience also showed that goats were very suitable animals in that slight symptoms were presented to our notice in a definite objective form. The lesser symptoms of caisson disease cannot be neglected, and there are reasons for supposing that their occurrence is not exactly conditioned by those experimental circumstances which in a more severe form produce serious and fatal results. They cannot be properly detected in mice or guinea-pigs or even in rabbits. Goats, while they are not perhaps such delicate indicators as monkeys or dogs, and though they are somewhat stupid and definitely insensitive to pain, are capable of entering into emotional relationships with their surroundings, animate and inanimate, of a kind sufficiently nice to enable those who are familiar with them to detect slight abnormalities with a fair degree of certainty.

The animals, 85 in number, used in the present experiments were a mixed collection of ordinary English goats of no particular breed. They

¹ The weights are only given in a few instances; from these it may be surmised that the dogs were small (5 to 12 kilos).

were about equally distributed between the sexes, and varied in weight from 10 to 30 kilos, the average being rather less than 20 kilogrammes. All were apparently adult, judging from the fact that none showed any increase in weight while in our possession. One or two (XIII A, XXXII A) seemed to be quite aged, but the rest were fully active.

On the whole the herd remained healthy. Two died of apical pneumonia and two of diarrhoea, which was at one time epidemic in a severe form. The cause could not be determined, but the trouble became much less marked after the animals were placed on a more meagre diet and corn withheld. Three animals were under some suspicion of being infected with *M. melitensis*¹; two of them seemed rather depressed (though not more so than appears to be natural in some goats), while the third showed no signs of ill-health. Various items of pathological interest were found in those which came to post-mortem: in the lungs various nematodes were found several times, *Linguatula* once, and a surgical needle once; a *Streptothrix* abscess in the stomach wall followed puncture with a needle to relieve distension; a bony tumour was found in an adrenal gland; in one old goat (XXXII A) the aorta was extensively atheromatous; flukes occurred in the liver once, while hydatids in the peritoneum were very common and intestinal worms abundant. None of these conditions (except possibly the arterial disease) can however be considered to have rendered the animals definitely abnormal as far as caisson disease was concerned, and none of them could be attributed to exposure to compressed air.

3. *Respiratory exchange of goats.*

The difficulties of measuring directly the circulatory activity of normal animals are almost insuperable. This must be however in general proportionate to the rate of respiratory exchange, and a number of determinations of the CO₂ production of our goats were made in order to get a line of comparison with other animals (and especially man) in respect of the rate at which air would be taken up by and discharged from the body.

¹ Of 22 animals whose blood was examined, 16 gave no reaction with *M. melitensis* at a dilution of 1:20, 3 gave some reaction at 1:20, while 3 animals gave complete agglutination up to 1:200 (XVIII A, XXVI A, XXIII A). Cultures from the blood during life were negative, and when they eventually came to autopsy cultures of blood, spleen, liver, inguinal, axillary, mesenteric and mammary glands were negative as regards *M. melitensis*. The exact history of these animals could not be obtained, but there is practically no doubt that they had never been out of England.

The observations were made by using the pressure-box as a respiration chamber. The animals were enclosed and hourly samples removed (after thorough mixing with an electric fan) and analysed in a delicate form of Haldane's gas analysis apparatus. The results were entirely satisfactory, the successive analyses showing a regular increase in the CO_2 . The goats led a regular life, and all the observations were made at approximately the same time of day so that they are fairly comparable with one another in respect of the influence of food. The animals remained fairly quiet, though they seldom lay down.

The results of 27 experiments are given in the next table. The analyses have been calculated in grammes of CO_2 per hour per kilo of

TABLE II.

Number of experiment	Temp. °C.	Bar. mm.	Duration hours	Pressure lbs. positive	Goats					CO ₂ gms. per hour		Remarks
					Number	Males	Females	Total weight kilos.	Average wt. kilos.	Per kilo body wt.	Per 1000 sq. cms. surface	
I	19	764	3	0	4	—	4	99.0	24.7	1.006	2.625	
II	13	763	2½	0	4	2	2	62.8	15.7	1.123	2.070	
III	15.5	765	1½	0	9	4	5	159.7	17.7	0.908	1.823	
IV	15	752	1½	0	9	3	6	171.1	19.0	0.727	1.749	
V	17	762	3	0	6	6	—	111.8	18.6	1.104	2.554	R. Q. 1.03.
VI	11	769	6	0	6	—	6	138.8	23.1	0.670	1.771	R. Q. 0.90.
VII	13	765	5	0	6	6	—	121.3	20.2	0.975	2.389	R. Q. 1.06.
VIII	13	754	4	45	7	7	—	142.7	20.4	0.887	2.187	
IX	13	740	4	45	8	—	8	193.7	24.2	0.627	1.682	
X	15	754	7	0	7	7	—	142.1	20.3	0.664	1.630	R. Q. 0.91.
XI	16	780	6	0	6	6	—	126.3	21.0	0.615	1.533	Fasting 20 hrs. : R. Q. 0.82.
XII	15	778	4	21	7	7	—	140.9	20.1	0.763	1.770	
XIII	15	774	7	0	8	—	8	193.7	24.2	0.548	1.469	R. Q. 0.82.
XIV	14	760	4	45	13	5	8	295.7	22.7	0.667	1.738	R. Q. 1.08.
XV	16	758	3½	25	6	6	—	127.9	21.3	0.959	2.367	
XVI	17	764	3¾	20	7	3	4	148.7	21.2	0.635	1.572	
XVII	15	762	4	0	6	6	—	127.9	21.3	0.669	1.652	
XVIII	17	762	1¾	45	6	—	6	122.1	20.3	1.020	2.504	
XIX	14	761	4	45	6	6	—	127.9	21.3	0.697	1.722	
XX	13	760	3	0	5	—	5	100.1	20.0	0.921	2.258	
XXI	16	760	4	45	5	—	5	100.1	20.0	1.104	2.701	
XXII	15	762	4	45	6	6	—	127.9	21.3	0.967	2.390	
XXIII	16	768	4	45	5	—	5	100.1	20.0	0.852	2.083	
XXIV	12	776	5	0	4	2	2	95.3	23.8	0.717	1.853	
XXV	14	775	5	0	4	2	2	95.3	23.8	0.751	1.941	
XXVI	14	775	5	0	4	1	3	76.9	19.2	0.624	1.501	
XXVII	13	766	5	0	4	1	3	76.9	19.2	0.704	1.693	

body weight and also per 1000 square centimetres of surface according to the usual formula $S \times 100 = \sqrt[3]{W^2} \times 11.2$, where S = surface in square centimetres and W the body weight in kilogrammes.

The goats used belonged to Series II (Exps. 1—4), III and IV.

The results of these experiments are very variable; the averages are shown in the next table:

TABLE III.

	No. of experiments	CO ₂ in grms. per hour	
		Per kilo body-weight	Per 1000 sq. cms. surface
At atmospheric pressure	16	0.795	1.907
At 45 lbs. positive	8	0.853	2.126
At 20, 21 and 25 lbs. positive	3	0.786	1.903
All pressure experiments	11	0.834	2.065
Males only	10	0.830	2.019
Females only	8	0.843	2.137
Mixed experiments	9	0.762	1.771
All experiments	27	0.811	1.971
		(410 c.c.)	(997 c.c.)

One may conclude that goats produce about 0.8 gm. CO₂ per kilo per hour under conditions of incomplete rest, and that no great departure from this figure is occasioned by the animals being under pressure up to 45 lbs. or by sex. It is shown elsewhere¹ that something more than 10% of the total CO₂ produced by goats comes from the fermentation of the contents of the alimentary canal, and figures detailed below (p. 409) indicate that one-fifth of the body weight is contributed by these contents. In comparing the CO₂ production of goats with that of man, we may regard these two corrections as roughly balancing one another and may neglect them.

It appears that man produces under conditions of bodily activity comparable to that of our experimental animals, about 0.45 to 0.5 gm. CO₂ per kilo per hour. Goats therefore show a respiratory activity approximately 1.7 times that of man. This figure corresponds fairly well with that calculated from the size. If the respiratory exchange per unit of surface is the same, a goat of 20 kilos will produce 1.5 times as much CO₂ per unit of weight as a man of 70 kilos.

4. *Method of conducting the experiments.*

No animals were subjected to experiment when obviously ill. As a rule five to eight animals were put in at one time. The pressure

¹ *Journal of Physiology*, vol. xxxvi. (1907), p. 283.

having been raised to the desired point, the chamber was entirely closed and no ventilation given until decompression began. The average CO_2 production of goats is about 435 c.c. per kilo per hour at ordinary temperatures. The chamber usually contained 100 to 150 kilos of goat so that the CO_2 rose about 0.45 to 0.55 % (measured at atmospheric pressure) per hour. In this way it never attained a harmful partial pressure in experiments lasting from a few minutes to four hours. In the few observations made with an exposure of eight hours, the CO_2 was allowed to accumulate for four hours and afterwards the chamber was ventilated so that the CO_2 did not exceed a partial pressure of 2 % of an atmosphere. No experiments have been made to directly examine the possible influence of CO_2 upon the incidence of caisson disease. It appears to the authors that the effect must (1) in any case be very slight with partial pressures of less than 2 or 3 %, and the result, if any, of the increased respiratory and circulatory activity must be in the direction of diminishing the ill-effects of decompression after any but quite short exposures¹.

After the preliminary experiments (Series I), the animals were never used more than once on the same day, and, with rare exceptions, not on succeeding days. In many cases indeed individual goats rested for a week or more between the experiments.

During decompression the animals could be watched fairly satisfactorily through the windows of the chamber, though fog of course completely blocked the view during the actual moments of rapid decompression. At the end they were allowed to escape from the chamber and run about free in the yard. They were kept under continuous observation for half an hour or longer, and were frequently seen throughout the day. We found that practically all the symptoms which were going to appear declared themselves within thirty minutes, though a few slight signs were probably missed. We also found that slight signs were much more obvious when the animals were not distracted or excited by food or other causes. During the breeding season it is advisable to keep the males and females separate, and, by removing any sources of interest, to allow the animals to fall into a state of meditative boredom. Under these circumstances, trivial symptoms are easily detected which are not made the subject of objective demonstration by animals engaged with their appetites.

¹ Greenwood (*British Medical Journal*, June 22nd, 1907, Supplement, p. 409) has recently found that high percentages of CO_2 do not increase the liability to decompression symptoms.

No observations were made of the temperature within the chamber during an experiment. Very hot and very cold weather did not seem to influence the results. The air in the chamber was always warmed by compression and sometimes also artificially, while decompression was of course accompanied by sudden, often very severe, spells of cold. No account has been taken of variations in atmospheric pressure. The extreme readings of the barometer on record are 806 and 689 mm. at sea level, and in this country 790 and 695 mm.¹, giving ranges of 117 mm. and 95 mm. or about $2\frac{1}{4}$ and $1\frac{4}{5}$ pounds. Even this variation, though it occurs at an important part of the absolute pressure scale, cannot be of great significance.

Times of exposure of one hour or less are, unless the contrary is directly specified, to be taken as indicating actual exposure to the given pressure, the time of compression (six minutes) being neglected. For longer exposures it was sometimes convenient to raise the pressure more slowly: in these cases therefore the times specified may indicate either the actual exposure *plus* four to six minutes compression or a virtual exposure calculated by adding the actual exposure to half the time of compression which is in minutes roughly one quarter of the pressure in pounds positive (see above, p. 362).

As will be gathered from the details given below, the general scheme of the experiments involved the examination of three variable factors—degree of pressure, duration of exposure and duration and mode of decompression. For the most part the degree of pressure was kept constant while the other two factors were varied. It soon appeared from the preliminary experiments that the individual variability of the animals was very large—larger indeed than the difference between many of the modes of decompression which it was desired to examine. It also appeared that the relative susceptibility of the different individual animals remained fairly constant so that after a time one could pick out goats which were known to be either susceptible above the average or definitely resistant to caisson disease. It was therefore clear that either an enormous number of animals had to be employed or the experiments had to be so framed as not to produce fatal results and so reduce the proportion of susceptible individuals in the herd. It appears probable that any 20 or 30 goats would give much the same results, but if many are lost it is necessary to discard the remainder and procure a fresh batch to be subjected to the comparative experience. Obvious reasons prevented this procedure. It was therefore necessary to be at

¹ *Nature*, vol. LXXV., 1907, p. 330.



“Bends” of fore-leg in a goat.

some pains to secure that the deaths should be as few as possible so that the same individuals might pass through a number of different combinations of pressure, exposure and decompression. The animals were therefore first put through the experiments which we surmised would give least symptoms, and were subsequently exposed to circumstances of progressively increasing severity. Even so, each batch of animals became selected to a more or less considerable degree. Be it noted however that one may in this way obtain strong evidence of an *a fortiori* kind. For, if the selected resistant members of the herd show many symptoms in the severest experiments, so much the more would the whole original lot of average animals have been affected. This individual variability of the animals renders many of our experiments incomplete, and should be constantly borne in mind in considering the results obtained.

5. *The symptoms observed in goats.*

The symptoms observed in goats in sequence to decompression are protean in character. The majority may however be grouped under a few definite heads.

1. *Bends.* The commonest symptom which we have observed consists of the exhibition of signs indicating that the animal feels uneasy in one or more of its legs. The limb, most commonly a fore-leg, is held up prominently in the air and the animal is evidently loth to bear weight upon it (see Plate VI¹). In mild cases such a limb is used normally in walking or running, but in other instances the animal limps more or less considerably when it is forced to use the affected member, and is often very anxious to lie down. No tenderness can be detected on pressure or manipulation of the leg and it is not altogether clear that the animal suffers definite pain. We have however noted that a goat may break its leg and immediately use it for progression without evincing any signs of pain. We may conclude from this that the response to stimuli which in many animals would be distinctly painful is largely suppressed in the goat to the level of the exhibition of a consciousness that the limb is somewhat abnormal and not well suited for active use. But it must be understood that this objective demonstration is a very conspicuous and definite symptom. There is little doubt that these symptoms observed in the legs of goats are the equivalent of the "bends" or "screws" which are the commonest

¹ We are indebted to Dr H. W. Armit for this photograph.

symptoms in caisson workers; in human experience they are of course accompanied by definite pain, often of a severe character.

The following table shows the distribution of "bends" in the last 110 cases observed:

TABLE IV.

One hind leg	28
One fore leg	70
Both hind legs...	1
Both fore legs	1
One fore and one hind leg	10
Total one leg	98
Total two legs	12
Total right	50
Total left	48

"Bends" may be seen immediately at, or indeed (but very rarely) shortly before the end of a long decompression. Most commonly however they come on after an interval of about 15 minutes; on the other hand they may be delayed still more. As might be expected, the period of delay varies with the duration of decompression: thus the average delay in a number of cases after rapid decompression (1 to 10 minutes) was 16 minutes, which was reduced by long decompression to six minutes. Their duration appears to be brief; all evidence of their presence has usually disappeared in one or two hours and it has been very exceptional for any trace of them to be present next day (16 to 20 hours).

"Bends" in parts of the body other than the limbs are very difficult to identify in animals; we have however occasionally noted symptoms which might well be bends in the trunk, though we are not prepared to definitely identify them as such.

2. *Temporary paralyses* may be of two kinds. In the first a general weakness is present accompanied with dyspnoea and there is dragging of the hind legs with foot-drop. These are clearly symptoms due to a general deficiency of oxygen from pulmonary embolism and are comparable to the paralyses seen in, *e.g.*, carbon monoxide poisoning in animals and men. In our records and the tables such cases are not classified as "paralysis" but as "dyspnoea." In the second group fall a series of cases which are obviously of nervous origin. The animal, while showing no signs of general illness, or in other instances having already had bends, exhibits foot-drop or a more extensive palsy in one or more hind- or fore-limbs. The paralysis does not usually come on till about 15 minutes after decompression, rapidly becomes more marked

for a few minutes after the first signs are noted, and then soon begins to mend, so that there is marked improvement in about half an hour, and by next day the animal is found quite well. This form of paralysis chiefly involves the hind legs (16 out of 19 cases).

3. *Pain.* In some cases the animals have shown signs of acute pain by urgent bleating and continual restlessness. Bleating in goats after decompression is usually a sign of distress such as is produced by cardiac and respiratory embarrassment and is often present in fatal cases. In other instances animals showing only severe bends bleat in a most distressing manner and are evidently in acute pain: at the same time they may gnaw at some part of their body (such as the testicles) as if localising the origin of the pain. In animals which have recovered, we have not had any instance where these signs persisted for more than 10 or 15 minutes.

4. *Permanent paralyses.* The onset is usually immediately after decompression, the condition is complete from the first and for at least several days there are no signs of improvement. In a few cases the first paralysis has passed off (to all appearances completely) in two or three hours and the animal has been found next morning to be again paralysed. This second paralysis is permanent. A similar history has often been noted in human cases. In 15 cases out of 16 the condition has been a paraplegia, and in one all four legs were affected more or less. In some there has been retention of urine, and one animal had to be killed on account of acute distension of the stomach which came on some 20 hours after the onset of the paraplegia. In the most severe cases the animals have been killed; others have however soon begun to mend and have lived for some months with a slight spastic paralysis of the hind legs.

5. A fair number of cases have occurred where the animal has been obviously ill, but in which it has been impossible to identify any definite local symptoms or any definite dyspnoea. The goat may lie down, refuse to move or to be tempted with corn (of which goats are inordinately fond), sometimes lying extended on the side, sometimes hurriedly rising, walking a few steps and then lying down again. On two occasions the most probable interpretation of the symptoms was that the animal was blind. The goat may run wildly about instead of becoming very apathetic and depressed. These and other such symptoms are on the whole somewhat persistent and the animal is often dull and poorly the next day. In one case (XXV A) the goat showed little but a marked apathy and distaste for food, but died 16 hours later.

6. *Dyspnoea* is usually the precursor of (7) *death* and only a minority of goats survived after showing clear dyspnoea. In these cases the condition has rapidly improved; more commonly however it progressively increases till the animal is moribund, when it is replaced by irregular, faint, gasping respiration. The mucous membranes become livid and pale and the animal lies for a short time unconscious before respiration stops. The heart continues to beat regularly throughout and the rate is not apparently much altered. Only on one occasion have we been able to hear gurgling in the heart on auscultation: it was then audible at some distance. Death ensues at varying periods after decompression; with very severe experiments (*e.g.* 100 lbs.: 1 hour: 1 minute)¹ it may follow in five or ten minutes: with more moderate conditions it is delayed for 20 or 30 minutes, or rarely for two or three hours: on three occasions it has followed still later, up to 40 hours. The delay in the onset of the first symptoms is often most striking; the animal may appear quite normal for as long as 10 or 15 minutes, dyspnoea then appears, the goat falls down helpless and in another 15 minutes is dead.

8. *Mechanical symptoms* are not important. We have not been able to satisfy ourselves that goats ever suffer materially during compression from the ear troubles which are so common in men. Abdominal distension is occasionally extreme, but the animal soon empties its distended stomach and seems to be little inconvenienced².

Our index throughout has been the presence of symptoms, not the presence of bubbles. Anticipating here a later section (p. 410) we may say we are in entire agreement with the view which attributes most of the severe symptoms of caisson disease to local or general blocking of the circulation by bubbles of gas. One might suppose in consequence that the incidence of severe symptoms, especially of paralyses, would be of a haphazard kind, since they would be to a large extent dependent on the chance distribution of bubbles by the blood stream. Some support for this view is perhaps to be found in the records of caisson workers given by von Schrötter; as far as can be ascertained from the details given, the cases of paralysis and dyspnoea were distributed through the whole range of pressure experienced by the men in about the same proportion to the total number of illnesses of all kinds, which latter increased greatly as the pressure became higher. It should however be noted that the range of pressure was small (up to 2·4 atmospheres positive), and

¹ *i.e.* pressure 100 lbs. positive; exposure for 1 hour; decompression in 1 minute.

² Post-mortem experience shows that the stomach alone is distended, not the bowels.

the general experience of caisson works as well as our own experiments with animals are distinctly at variance with these results. We have noted only two instances of what may be called "chance incidence" of paralysis: (a) goat XV (Series II) had a paraplegia after 15 minutes' exposure at 75 lbs. and decompression in 30 minutes uniformly, and afterwards lived for some time, passing through much more severe experiments without symptoms, and eventually being killed with some difficulty by 75 lbs: 2 hours: 1 min.: (b) goat XII A (Series III) after 45 lbs.: 1 hour: 10 min. uniform, had apparently very severe bends; it did not however recover in the usual way and became partially paraplegic, subsequently passing through many comparatively severe experiments without symptoms. The tables of our results seem to show quite clearly that as the conditions of experiment become more searching, not only does the frequency of symptoms increase but the proportion of severe to total symptoms becomes much greater.

It is necessary for comparative purposes to form some idea of the relative importance of these different symptoms, and to consider how far they may be classed as relatively dangerous or comparatively negligible. "Bends" are clearly a slight symptom; there is abundant evidence both in goats and men that their occurrence is no indication of urgent danger to life. At the other end of the scale we have death. Dyspnoea is not far removed in significance from death, and lasting paralyses are somewhat less serious than dyspnoea. Next in order come pain and those indeterminate conditions which we have grouped as "indefinite and various general": these may be followed by death and are much more indicative of danger than bends. Temporary paralyses are not so important and we are inclined to the view that they are not much more dangerous than bad bends. This classification is based for the most part on our experience as to the kind of experiment with which each group of symptoms is commonly associated, and the way in which the different groups are associated together in the same experiment. The individual variability of the animals introduces many difficulties, but it is certain that the more severe the conditions of pressure, exposure and decompression, the more likely it is that the animals will suffer from symptoms which we have classed as severe.

Immunity to symptoms. There is not the slightest ground, either theoretical or experimental, for supposing that animals or men, as the result of repeated exposure to compressed air, acquire any immunity to the formation of bubbles within their persons. It must be remembered in this connection that the susceptible individuals become eliminated,

so that those who have been through many decompressions necessarily show more than average resistance. The matter is not so clear with regard to the exhibition of symptoms resulting from such bubbling. We have a certain amount of evidence, too vague to be detailed, that some goats show slight bends rather more easily in their first few experiences, and it is not difficult to imagine that they might grow to neglect altogether those bubbles which evidently cause them no very great inconvenience at any time. With severe symptoms it is of course different: no one can suppose that a goat acquires immunity to extensive pulmonary air emboli or to infarction of the spinal cord.

TABLE V.

Series I. June—July 1906. Pressure 75 lbs. positive (=6 atmospheres absolute). Compression in 39—41 minutes. The details of the stage decompressions are shown in Table IX.

		Decompression minutes40—50 uniform			40—50 stages		
Actual exposure minutes.....		12	15	30	12	15	30
No. of goat	Sex						
I	M	0					
Pa	M	0	bends	0		0	0
XIII	F	bends					
XVIII	F	bends	0	0			0
		+ indefinite ¹					
XXI	F	0+0 ¹	bends				
XXII	F	0	bends				
X	F		bends	bends			0
XVI			bends	bends		0	
XX	F		bends	bends	bends		
XXIV	M		0	bends		bends	0
XXVII	F		0			0	0
XXIX	F		0	bends, dyspnoea		0	
XXX			bends			bends	
XXXII			bends				
III	M			0			0
IV	M			0			bends
VI	M			0			0
XXVI	M			bends			bends + bends ¹
XXVIII	F			bad bends			
VIII	M				0		
XIV					bends		
XIX					bends		
II	M						bends
XXV	M						bad bends

¹ Each of these experiments was repeated upon the same animal: both results are shown. XVIII was generally uneasy, lay down, nothing definite.

TABLE VII.

Series III. February to June 1907. Pressure 45 lbs. positive (4 atmospheres absolute). The details of the stage decompressions are shown in Table IX.

Exposure minutes		15		30		45		60		90		120		240		480	
Decompression minutes	No. of goat	2		30		30		10 uni-form		30 stages		10 uniform		10 uni-form		62 stages	
		Weight kilos	Sex	2	30	30	30	10 uni-form	30 stages	30 stages	30 stages	10 uniform	30 stages	10 uni-form	62 stages	10 uni-form	62 stages
3	M	24.3	M	0	0	0	0	bad bends	0	0	0	0	0	0	bends	0	0
4	M	19.6	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0
X A	M	17.0	M	0	0	0	0	0	0	0	0	temp. paral.	0	bends	0	temp. paral.	0
XI A	M	20.0	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0
XII A	F	19.2	F	0	0	0	0	para-plegia ² bends	0	0	0	0	0	0	bends	temp. paral.	0
XIII A ¹²	F	28.2	F	0	0	0	0	0	0	bad bends	0	bends	0	0	0	temp. paral.	0
XIV A ⁸	M	16.4	M	0	0	0	0	bends	0	sl. bends	0	0	0	0	bends	0	0
XV A	F	15.5	F	0	0	0	0	0	0	obscure ³	0	bends	0	bends	bends	bends	0
XVI A	M	21.1	M	0	0	0	0	bends	0	0	0	0	0	temp. paral.	0	0	0
XVII A ⁹	F	26.8	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0
XVIII A ¹¹	F	32.0	F	0	0	0	0	0	0	0	0	0	0	bends	0	bends	0
XIX A	M	22.4	M	bends	0	0	0	0	0	0	0	bends	0	0	0	0	0
XX A ¹⁰	F	19.8	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0
XXI A	F	16.0	F	0	0	0	0	bends	0	0	0	bends	0	bends	bends	bends	0
XXIII A	F	26.3	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0

¹ Six other animals had this experience also, of which 7, XXVII A, and XXIX A had bends, and XXIV A, XXX A and 9 showed no symptoms. ² Never so completely paralysed that it could not walk, but no improvement occurred during 21 weeks. ³ Bleating, seemed lost and walked into objects as if blind: well in 2 hours. ⁴ Acute distension of stomach developed next day; goat killed. ⁵ Killed next day. ⁶ Hind legs and, to a less degree, fore legs also: could walk in 3 days; killed 15 days when much improved. ⁷ Killed 3 days. ⁸ Broke leg, killed. ⁹ Died of pneumonia: no reason to connect this with decompression. ¹⁰ Died, cause unknown. ¹¹ 75 lbs., 3 hours, 58 seconds: died 12 minutes without showing dyspnoea or any symptoms except collapse. ¹² Apparently elderly.

6. *Results of goat experiments.*

The detailed results of the experiments on goats are set out in the accompanying tables. With the exception of those in Series I, these tables contain nearly all the experiments which were made. Read vertically the columns give the results of the different combinations of pressure, exposure and decompression: the records of individual goats can at the same time be read on the horizontal columns. The tables give however no indication of the chronological sequence of events. In Series I only a few experiments are given; the series actually comprised 164 experiments on 34 animals, but the procedures adopted were, through ignorance, so ill-devised that no very definite results were obtained, though we gained information which enabled us to devise more satisfactory experiments subsequently. We have therefore extracted from Series I only a few results which illustrate the difference between stage and uniform decompression. The four series roughly represent four batches of goats, except that the animals of Series IV are the remnant of Series III with the addition of a further small herd. When reference is made to individual goats of Series I, the series is noted; otherwise the goats of Series III and IV are distinguished by "A": this does not apply to hornless animals which are specified by Arabic instead of Roman numerals, no two goats having the same number.

TABLE IX. *Showing the decompressions of goats from 75 lbs. and 45 lbs. Times given in minutes, one minute being occupied in each drop after the first.*

Series	Decompressions from 75 lbs. +								Decompressions from 45 lbs. +			
	I	II	II	II	IV	IV	IV	IV	III	III	III	III
Columns..	4, 5, 6	4, 5, 8, 9, 11, 12, 13, 16	4 (part)	14	12	7	10	9	3, 4, 7, 9 12, 15	8	13	16
First drop in	4	4	5	2	3	3	3	4	1	2	2	2
Wait at 32							3					
„ 27	5	2		7	15	4	9	2				
„ 22½	4			5	19	4	14	4				
„ 18	4	2	5	5	19	9	14	16		4	9	14
„ 14	4	4	4	10	24	9	14	16	3	14	14	14
„ 9	4 or 9	4	9	15	24	14	14	19	9	14	14	14
„ 4½	10 or 14	10	9	20	24	19	14	19	14	14	14	14
Total time	41 or 50	31	36	70	134	68	92	86	30	52	57	62

In the next table (Table X) the results are condensed and grouped in a simpler way, and one or two more experiments are given from Series I.

TABLE X.

Pressure lbs. positive	Compression minutes	Actual exposure minutes	Decompression minutes	No. of goats	No symptoms		Bends				Temporary paralysis	Various indefinite	Paraplegia	Dyspnoea	Total severe symptoms	Death
					Number	Percent.	Slight	Bends	Bad	Total						
75	6	1	60 un.	8	7	87		1		1					0	0
		1	1	6	6	100				0					0	0
		3	1	5	4	80				0		1			1	0
		3	10 un.	2	2	100				0					0	0
		6	1	6	6	100				0					0	0
		10	1	7	6	86				0			1		1	0
		15	1	6	2	33	1	1		2	1				1	1
		15	10 un.	7	2	29		3		3		1			1	1
		15	31 st.	34	29	85	2	2	1	5					0	0
		15	31 un.	36	19	53	3	8		13		1	2		3	1
		15	90 un.	12	9	75		3		3						
		30	31 st.	23	12	52		7	1	8	3				3	0
		30	31 un.	6	1	17		3	1	4	1				1	0
		30	68 st.	14	14	100				0					0	0
		30	68 un.	14	7	50		7		7					0	0
		60	31 st.	22	15	68		3	1	4	1	1		1	3	0
		120	31 st.	9	0	0		4	3	7		1			1	1
		120	70 st.	14	9	64		4		4				1	1	0
		120	70 un.	13	4	31	1	6		7	1		1		2	0
		120	92 st.	19	15	79		3		3	1				1	0
		120	100 un.	19	10	53		1	2	3	2	1		2	5	1
		180	134 st.	14	12	86		2		2					0	0
		180	134 un.	10	5	50	1	1	3	5					0	0
		240	31 st.	8	2	25		3	1	4	1				1	1
		240	31 un.	4	0	0			2	2	1				1	1
51	6	180	4	10	2	20		2	1	3		1		2	3	2
45	6	15	2	15	14	93		1		1					0	0
		30	2	15	12	80		3		3					0	0
		45	30 st.	14	14	100				0					0	0
		60	1	13	10	77		4		4					0	0
		60	10 un.	13	7	54		4	1	5			1		1	0
		60	30 st.	13	9	69	1	3		4					0	0
		60	52 st.	13	10	77	2	1		3					0	0
		90	30 st.	8	5	62		1	1	2		1			1	0
		120	1	10	4	40		1	1	2	1		3		4	0
		120	10 un.	12	6	50		4		4	1			1	2	0
		120	30 st.	13	12	92		1		1					0	0
		120	57 st.	15	13	87		2		2					0	0
		240	10 un.	11	6	55		4		4	1				1	0
		240	30 st.	13	11	85		2		2					0	0
		240	62 st.	15	9	60		6		6					0	0
		480	10 un.	11	6	55		3		3	2				2	0
30	6	60	10 un.	19	15	79		4		4					0	0
25		240	2	23	21	91		2		2					0	0
20		240	2	22	21	95		1		1					0	0
75	39	1	7 un.	8	7	87				0			1		1	0
		10	10 un.	4	3	75				0					0	1
		12	45 st.	4	1	25		3		3					0	0
		12	45 un.	6	4	67		2		2					0	0
		15	45 st.	6	4	67		2		2					0	0
		15	45 un.	12	4	33		8		8					0	0
		30	45 st.	11	7	64		3	1	4					0	0
		30	45 un.	12	5	42		5	1	6			1		1	0
		30	10 un.	4	0	0				0					0	4
		30	10 un. ¹	4	4	100				0					0	0
		30	10 un. ²	4	0	0		2		2			1		1	1
		60	75 un.	4	2	50		1		1					0	1

¹ Recompressed at once to 15 lbs. for 32 minutes.² Recompressed to 15 lbs. for 37 minutes 18 minutes after decompression.

un. = uniform decompression : st. = decompression by stages.

The following tables give in the simplest form the experimental evidence on certain points which are of especial importance.

(I) *Experiments showing that a certain minimum pressure is required to give symptoms in goats, and that the results vary with the pressure.*

TABLE XI.

Pressure in lbs. positive	Exposure in minutes	Decompression in minutes	No. of goats	No symptoms	Bends	Severe symptoms	Death
20	240	2	22	21	1	0	0
25	240	2	23	21	2	0	0
30	60	10 uniform	19	15	4	0	0
45	60	10 „	11	7	3	1	0
60 ¹	45	15 „	4	1	3	0	0
75	15	31 „	36	19	13	3	1
75 ²	50	10 „	4	0	0	0	4

¹ Experiment in Series I: compression 30 minutes, exposure 30 minutes.

² Series I: compression 40 minutes, exposure 30 minutes.

These experiments show that the effects become more severe as the pressure increases although the duration of exposure was at the same time diminished and the duration of decompression increased. It was necessary to arrange the experiments in this way to prevent an inconvenient mortality among the animals.

(II) *Experiments showing that the duration of exposure to high pressures is of great importance.*

TABLE XII.

Pressure 75 lbs. positive, reached in 6 minutes.

Exposure in minutes	Decompression in minutes	No. of goats	No symptoms	Bends	Severe symptoms	Death
1	1	6	6	0	0	0
3	1	5	4	0	1	0
6	1	6	6	0	0	0
10	1	7	6	0	1	0
15	10 uniform	7	2	3	1	1
15	31 stages	34	29	5	0	0
30	31 „	23	12	8	3	0
60	31 „	22	15	4	3	0
120	31 „	9	0	7	1	1
240	31 „	8	2	4	1	1

These experiments show that goats have taken up enough air in 15 minutes to give severe symptoms on decompression in 10 minutes, while, if the exposure is less than 10 minutes, nearly all the animals escape, even with sudden decompression. Note too that with short

exposures and rapid decompressions such symptoms as appear are more frequently severe, and that bends are proportionately less common than with longer exposures and slower decompressions. Beyond 15 minutes exposure the results are somewhat irregular, but on the whole there is a progressive increase of bad symptoms up to two hours exposure. The results after four hours exposure are about the same, but the animals used (see Table VI, Series II) were to a large extent selected by previous experiments, so that it would appear that goats are practically saturated in about three hours¹.

TABLE XIII.

Pressure 45 lbs. positive.						
Exposure in minutes	Decompression in minutes	No. of goats	No symptoms	Bends	Severe symptoms	Death
15	1	15	14	1	0	0
30	1	15	12	3	0	0
60	1	14	10	4	0	0
120	1	10	4	2	4	0
60	10 uniform	11	7	3	1	0
120	10 „	11	6	4	1	0
240	10 „	11	6	4	1	0
460	10 „	11	6	3	2	0

These figures show that with a duration of exposure up to about three quarters of an hour, no severe symptoms follow even sudden decompression. The series with sudden decompression shows that the results after two hours are much worse than after one hour. This is not clear from the series with 10 minutes decompression, which, however, show that the results do not become distinctly worse even after

¹ The following figures have been compiled from the records of Heller, Mager and von Schrötter as illustrating the saturation time for dogs of about 10 (?) kilos. The corresponding data for other animals do not seem to have been determined. Pressure 62–69 lbs., compression in 5–16 minutes, decompression $\frac{1}{2}$ to 1 minute.

Exposure minutes	Number of experiments	No symptoms	Mild paralysis and bends	Lasting paralysis	Paralysis and asphyxia	Asphyxia
Less than 10	1	1	0	0	0	0
10–29	6	5	1	0	0	0
30–59	12	0	6	2	3	1 (lived)
60–120	19	2	2	2	3	10

Four of the group “paralysis and asphyxia” died, and the other two would probably have died if they had not been killed. All but one in the “asphyxia” group died, but in none of the rest was the decompression immediately fatal. These results seem to show pretty clearly that dogs require more than an hour to become saturated. It is strange that the authors conclude (*Luftdruckerkrankungen*, p. 806) that saturation is so far complete in about 38 minutes *in man* that no further intake of nitrogen is of any practical importance.

eight hours exposure. Note that of the nine severe symptoms, five were temporary and four permanent paralyses: only one case of dyspnoea was seen in the whole of the experiments at 45 lbs. (Series III) and the one case of severe illness of obscure nature was suggestive of temporary local cerebral anaemia. At 75 lbs., out of 26 severe cases, four had dyspnoea, four permanent and 12 temporary paralysis, and six indefinite: seven died.

(III) *Experiments to show that the duration of decompression is of great importance.*

TABLE XIV.

Pressure 75 lbs. positive, reached in 6 minutes.

Exposure	Decompression	No. of goats	No symptoms	Bends	Severe symptoms	Death
15	10 uniform	7	2	3	1	1
15	31 „	36	19	13	3	1
15	90 „	12	9	3	0	0
30	31 stages	23	12	8	3	0
30	68 „	14	14	0	0	0
120	31 „	9	0	7	1	1
120	92 „	19	15	3	1	0

(IV) *Experiments to show that the absolute range of pressure through which decompression occurs may be of less importance than the relative range of absolute pressure.*

TABLE XV.

Pressure in lbs. +	Exposure in minutes	Decompression to lbs.	Fall of pressure in lbs.	Relative reduction of absolute pressure	Duration of decompression in minutes	No. of goats	No symptoms	Bends	Severe symptoms	Death
75	180	+24	51	2.3:1	1½	10	10	0 ¹	0	0
51	180	0	51	4.4:1	4	10	2	3	3	2
45	120	-6	51	6.7:1	6	3	0	1	1	1
39	120	-6	45	6.0:1	6	4	1	0	3	0
45	120	0	45	4.0:1	1	10	4	2	4	0

¹ There were three cases of bends at the ultimate end of a two hours' decompression.

A sudden drop of about 50 lbs. from 75 lbs. positive to 27 or 24 lbs. positive has been made about 200 times altogether in the course of these experiments without producing any symptoms, and about two-thirds of the animals showed no symptoms at the end of the stage decompression. The animals were however only left a short time at 27 lbs. before proceeding with the further decompression. In the

present series the animals were left for one hour at 24 lbs. and watched very carefully, and afterwards suddenly decompressed to 17 lbs. and again observed for half an hour. The same goats were subsequently dropped suddenly from + 51 lbs. to atmospheric pressure with very disastrous results, and a drop of 51 lbs. from + 45 lbs. to - 6 lbs. was even worse. The details of these experiments are given in Appendix III. Owing to the cooling effect of rapid decompression, the falls from + 45 and + 39 to - 6 lbs. were interrupted by a delay of about two minutes at atmospheric pressure so that they were in a rough way stage decompressions.

(V) *Experiments showing the importance of the mode and spacing of decompression.*

The next table shows in brief the results of seven groups of experiments undertaken with the purpose of directly testing the results of stage decompression in comparison with those of uniform decompression in the same total time. The only exceptions to the parallelism of the experimental conditions are (1) in group ζ the time of uniform decompression was extended from 92 to 100 minutes in order that it might correspond to the supposed safe rate of 20 minutes an atmosphere¹; and (2) in group β stage decompression, three animals were decompressed by stages in an abnormal way (see Table VI and note, Series II); since these stages were certainly not more favourable to the animals than those used for the rest of the group, we have included the results.

In considering these results it must be clearly understood that the stage decompressions used were not in most cases intended to be safe for the particular exposure to which they were attached. The only two groups which were intended to be safe (δ and η) gave fairly satisfactory results; with 30 minutes exposure (+ 6 minutes compression) at 75 lbs. and 68 minutes stage decompression, we obtained no illnesses in 14 goats, and with three hours exposure and two and a quarter hours stage decompression only two cases of bends in the same number of animals. For comparative purposes it was desirable that the stage decompressions should produce symptoms of some kind, and they were intentionally designed so to do in so far as our knowledge allowed².

¹ The details of the experiments in this group are given in Appendix III.

² The stage decompressions from 45 lbs. pressure are likewise all shorter than what we calculate to be safe. The stoppages are also imperfectly spaced. The proper spacing and duration of stoppages could not be calculated till the results of the experiments were known, and we realised the extreme slowness of saturation and desaturation.

TABLE XVI. *Showing the comparison between the results of stage and uniform decompression. Pressure 75 lbs. positive.*A. *All experiments.*

Group	Exposure minutes ¹	Decompression, minutes	Decompression, method	Number of animals	Number of decompressions	No symptoms	Per cent.	Bends				Total	Temporary paralysis	Various general	Paraplegia	Dyspnoea	Total severe	Death
								Doubtful	Slight	Bends	Bad							
<i>a</i>	12-30	45	stages	18	22	12	55			9	1	10					0	
<i>β</i>	15	31	"	18	34	29	85		2	2	1	5					0	
<i>γ</i>	30	31	"	15	23	12	52			7	1	8	3				3	
<i>δ</i>	30	68	"	14	14	14	100					0					0	
<i>ε</i>	120	70	"	14	14	9	64			4		4				1	1	
<i>ζ</i>	120	92	"	19	19	15	79			3		3	1				1	
<i>η</i>	180	134	"	14	14	12	86			2		2					0	
Total					140	103	74	0	2	27	3	32	4	0	0	1	5	0
<i>a</i>	12-30	45	uniform	19	32	14	44			15	1	16		1		1	2	
<i>β</i>	15	31	"	18	36	19	53	2	3	8		13		1	2		3	1
<i>γ</i>	30	31	"	6	6	1	17			3	1	4	1				1	
<i>δ</i>	30	68	"	14	14	7	50			7		7					0	
<i>ε</i>	120	70	"	13	13	4	31		1	6		7	1		1		2	
<i>ζ</i>	120	100	"	19	19	10	53			1	2	3	2	1		2	5	1
<i>η</i>	180	134	"	10	10	5	50	1	1	3		5					0	
Total					130	60	46	3	5	43	4	55	4	3	3	3	13	2

B. *Experiments on identical animals.*

<i>β</i>	15	31	stages	18	34	29	85		2	2	1	5					0	
<i>γ</i>	30	31	"	6	6	4	67			2		2					0	
<i>δ</i>	30	68	"	14	14	14	100					0					0	
<i>ε</i>	120	70	"	13	13	9	69			4		4					0	
<i>ζ</i>	120	92	"	19	19	15	79			3		3	1				1	
<i>η</i>	180	134	"	10	10	8	80			2		2					0	
Total					96	79	82	0	2	13	1	16	1	0	0	0	1	0
<i>β</i>	15	31	uniform	18	36	19	53	2	3	8		13		1	2		3	1
<i>γ</i>	30	31	"	6	6	1	17			3	1	4	1				1	
<i>δ</i>	30	68	"	14	14	7	50			7		7					0	
<i>ε</i>	120	70	"	13	13	4	31		1	6		7	1		1		2	
<i>ζ</i>	120	100	"	19	19	10	53			1	2	3	2	1		2	5	1
<i>η</i>	180	134	"	10	10	5	50	1	1	3		5					0	
Total					94	46	49	3	5	28	3	39	4	2	3	2	11	2

¹ Group *a* compressed in 39 minutes; the rest in 6 minutes or, with long exposures, in 39 minutes and half the time of compression deducted from the actual time of exposure.

The results are given in two forms: (*A*) shows the fate of all the animals tested to obtain the direct comparison between stage and uniform decompression, while in (*B*) the figures are confined to the effects (in the same experiments) on animals which were exposed to both stage and uniform decompression in each group. This emendation removes the only very severe symptom and three out of four of the temporary paralyses caused by stage decompression. Goat XXI (Series II) was advancing in pregnancy and, after having nearly died as the result of quick stage decompression, was excluded from the experimental troupe; the effect of the corresponding uniform decompression on this animal can therefore be only surmised. The effects of 31 minutes stage decompression after 30 minutes exposure were so bad that, not wishing at this stage to risk losing any animals, the parallel experiment with uniform decompression was limited to the more resistant animals. In group β (*B*) two of the animals were only decompressed once by stages. One had died from uniform decompression, and the other had broken a leg and had to be killed.

The figures show that the ratio of animals showing no symptoms with stage decompression to those escaping after uniform decompression in the same total time is about eight to five. Be it noted too that the difference between the two methods is in the same sense, *i.e.* in favour of stage decompression, in each of the seven groups, including group α (Series I) where the stages were less well arranged than afterwards. The difference between the two methods appears still more strikingly in the quality than in the quantity of the symptoms produced. For while but one animal had symptoms which can be called distinctly severe after stage decompression, as many as eleven were materially ill after the corresponding uniform decompressions, and one died.

This difference may perhaps obtain more definite expression if we assign numerical values to the different symptoms. Making bends = 1, temporary paralysis = 2, and so on up to death = 6, we obtain the following results, showing a ratio of nearly five to one (*B* grouping) in favour of stage decompression :—

Group	Stages	Uniform
β	5	30
γ	2	6
δ	0	7
ϵ	4	13
ζ	5	26
η	2	5
Total	18	87

This method is of course very rough. "Death" is worth more than six times "bends," and bends should have different values according to the sort of experiment. Bends arising from short exposures and relatively rapid decompressions (*e.g.* group β) indicate that the exposure has been long enough to allow material saturation and are very significant, while if bends show merely the extreme slowness with which the tissues in which they arise get rid of the excess gas (*e.g.* group η), they are of much less moment.

If we exclude bends, and count only the more serious symptoms, or death, the comparison becomes still more striking, the ratio being then two for stage decompression, as compared with 50 for uniform decompression.

(VI) *Experiments illustrating the difference between different kinds of animals.*

(1) Five goats (XXIV A, XXVI A, XXVII A, 7 and 9), 10 small guinea-pigs (175 to 275 gms., average 230 gms.), 9 mice (average 20 gms.), 12 small rats (average 35 gms.), 9 medium rats (average¹ 85 gms.), 8 large rats (average¹ 200 gms.), and 4 rabbits (1285, 1450, 1850, 2850 gms.) were compressed to 72 lbs. in 7 minutes and left at that pressure for 3 more minutes and decompressed in 50 seconds. Goat 9 had a curious short seizure and rolled over on the ground 10 minutes after decompression; it seemed alright immediately afterwards and showed no after effects. One small rat became paraplegic at once, and two other small rats were found dead next morning; one of these had bubbles in the heart. The rest of the animals showed no symptoms. The incidence of illness on the young rather than on the old rats is curious in view of the demonstration of the general immunity of young animals by Hill and Greenwood²: it was perhaps correlated with the shortness of the exposure.

(2) Twelve small rats, 13 medium rats, 8 large rats, 59 mice, 7 rabbits, 10 guinea-pigs, and 1 old hen were raised to 72 lbs. in 10 minutes, left for 1 hour and then decompressed in 50 seconds. No goats were put in since it was well established that this experience would have killed all of them. The hen and the largest rabbit (weight 2800 gms.) died in 5 minutes, and 1 guinea-pig became paraplegic in 10 minutes and died in 20 minutes. All three were extensively

¹ The details were eaten by a goat. All the animals were about the same size.

² *British Medical Journal*, June 22nd, 1907, Supplement, p. 408.

bubbled; it is interesting to note that there were no bubbles in the avascular eggs of the hen. None of the other animals showed any symptoms.

(3) Five rabbits, 10 guinea-pigs, 23 mice, 10 small, 9 medium and 6 large rats were compressed to 51 lbs. in 7 minutes, left there for 2 hours 56 minutes and decompressed in 45 seconds. In similar experiments, out of 10 goats 2 died and only 2 escaped without symptoms (see above, p. 398). The largest rabbit, a very fat animal weighing 2·9 kilos, died 9 minutes after decompression: the rest showed no symptoms.

(4) Six goats (3, XIA, XIIA, XXIA, XXIVA, XXVIIA), 7 guinea-pigs and six rabbits were raised to 75 lbs. in $5\frac{1}{2}$ minutes, left for 15 minutes and decompressed in 42 seconds. Goat XXVIIA had dyspnoea and paraplegia and was found dead next morning: XXIVA had temporary paralysis of both hind legs without dyspnoea and was quite recovered in an hour: 3 and XXI A had bends, while XI A and XII A showed no symptoms. None of the small animals were affected.

(5) Seven goats (3, X A, XI A, XII A, XXI A, XXIVA, XXVII A), 7 guinea-pigs, 5 rabbits, 7 medium and 12 large rats and 37 mice were compressed to 75 lbs. in 6 minutes, left 10 minutes and decompressed in 48 seconds. Goat X A had paraplegia. The other goats and the small animals showed no symptoms.

(6) Guinea-pigs, mice and rats were compressed with ourselves to 30 lbs. in 15 minutes, and 1 guinea-pig, 1 mouse, 1 medium and 1 large rat were killed with chloroform after 33 minutes. After decompression in 26 minutes by stages, many bubbles were found in the heart and vessels of the guinea-pig, a few in the mouse's heart, a few in the great vessels of the large rat, but none in the medium sized rat.

7. *Individual variation among the experimental animals in their susceptibility to decompression symptoms.*

The variation in the individual susceptibility of different goats is very marked. The same variation has been noted constantly among both divers and caisson workers, and is apparent in most of the published animal experiments. As an example, the following figures have been extracted from the tables of experiments at 75 lbs. All four animals were males and very similar to one another in all obvious respects: two were resistant and two susceptible.

TABLE XVII.

Exposure	Decompression	XIII (17·8 kg.)	X (16·4 kg.)	2 (16·2 kg.)	XV (16·8 kg.)
15 mins.	31 mins. uniform	0	0	slight bends	paraplegia
15 „	31 „ „	0	0	paraplegia	0
15 „	31 mins. stages	0	0	0	slight bends
15 „	31 „ „	0	0	bends	0
30 „	31 „ „	0	bends	pain, temporary paralysis	bends
60 „	31 „ „	bends	0	0	0
120 „	70 mins. uniform	0	bends	paraplegia	bends
120 „	70 mins. stages	0	0	bends	bends

In all, therefore, goats X and XIII showed mild symptoms three times in 16 decompressions, while in the same experiments goats 2 and XV showed symptoms 11 times, and on 4 occasions these were of a severe character.

It might be supposed that this variation was only in the exhibition of symptoms, depending on individual susceptibility to pain, &c., and did not represent a variation in the amount and distribution of bubbles within the body. But post-mortem experience shows that the amount of bubbling present in two animals killed in the same experiment may be very different; and in living animals it is clear that on the whole susceptibility to bends involves susceptibility also to the more severe symptoms, which cannot be much altered by the temperament of the animal.

The complete explanation of this individual variation in susceptibility probably requires a knowledge of the details of caisson disease far beyond that which we at present possess. Data exist, however, on which the influence of several factors may be discussed.

(A) *Influence of sex.* The following table shows the sum of the results of the experiments grouped according to the sex of the animals. The groups defined as "selected" include only those experiments in which the animals examined were approximately representative: in Series II for example the figures given are the totals of those experiments in which 10 or more animals were examined, while in Series III and IV are summed only those observations which included both sexes about equally (Series III, expts. 1, 2, 5—8, 10—17, Series IV, expts. 18—20).

It would appear from this that there is no clear difference between the sexes in liability to decompression symptoms in general. The experiments suggested however that under certain circumstances there might be a marked difference in the susceptibility to death. In

Series I, of 5 deaths, 3 were in females, a distribution of fatalities corresponding to the numbers of the sexes (males 12, females 16) used, while in Series II are shown 1 death in 7 males and 4 deaths in 11 females. All these last four animals were to some degree advanced in pregnancy, and their mortality is very probably to be associated with this condition, which, in the goat, is accompanied by a marked increase in the subcutaneous and intra-abdominal fat. That the deaths in Series I did not fall more heavily upon the females is perhaps to be correlated with the fact that these experiments were made in the summer and none of the goats were found pregnant, while the autopsies of Series II showed that in the winter practically every female is pregnant.

TABLE XVIII.

	Males				Females			
	Number	Decom- pressions	Illnesses	Per cent.	Number	Decom- pressions	Illnesses	Per cent.
Series I :								
Total	12	78	25	32	16	71	35	49
Series II :								
Total	7	84	42	50	11	91	38	42
Selected	7	64	26	41	11	79	27	34
15 mins. } exposures }	7	28	9	32	11	42	13	31
1 and 2 hrs. } exposures }	7	25	11	44	8	24	10	42
Series III and IV :								
Selected	7	108	26	24	8	113	29	26
Total	26	270	93	34	35	275	102	37

Influence of size. In the same way the influence of size on susceptibility may be examined. In the next table the animals are grouped as above and below the average weight for each sex.

TABLE XIX.

	Above average weight			Below average weight		
	Decompressions	Illnesses	Per cent.	Decompressions	Illnesses	Per cent.
Series II :						
Selected	55	22	40	80	26	32·5
15 mins. ex- } posure }	27	9	33	39	9	23
1 and 2 hrs. } exposure }	20	9	45	26	12	46
Series III and IV :						
Selected	102	19	19	119	36	30
Total	157	41	26	199	62	31
Journ. of Hyg. viii						26

The results are contradictory unless (which appears hardly possible) there is an essential difference between exposures to high (75 lbs.) and to low (45 lbs.) pressures. The sums of the whole show no material difference between large and small goats. Theoretically, with decompressions of moderate length such as were used in the experiments under consideration, small goats should be somewhat more susceptible than large goats with short exposures since they should saturate more quickly in proportion to their relatively greater gaseous exchange¹. This is not borne out by the experiments, in which however the rate of decompression may not have been quick enough to bring out the difference. It is, on the other hand, obvious that the larger goats should be more susceptible after long exposures with any except very short or very long decompressions: this is confirmed by the experiments at 75 lbs. (Series II), but those at 45 lbs. (Series III) show a greater difference in the opposite sense.

In comparing the incidence according to sex with those arranged according to weight, it will be noted that in Series II the males are somewhat more susceptible though they are rather smaller, while the same experiments, arranged by weights, show that the heavier animals suffer more frequently. In Series III, in which the male group is again composed of smaller animals, the susceptibility of the sexes is equal, while the lighter animals are more susceptible if weight be taken as the criterion. The only conclusion to be drawn is that these figures do not indicate that either sex or weight was a determining factor in the incidence of decompression symptoms.

Influence of the activity of gaseous exchange. General considerations suggest rather strongly that the susceptibility would be found to vary with the activity of gaseous exchange, directly as regards short exposures and inversely as regards long exposures. In most of our experiments, especially those of Series III, the incidence of symptoms has been conditioned rather by the mode of decompression than by the duration of exposure. As a whole, then, the goats with the most active exchange should prove to be the least susceptible.

The respiration results already given have been analysed in reference to this point: the results are variable and inconclusive and need not be detailed. This is perhaps not very remarkable when we consider that the animals were not grouped for the respiration experiments according

¹ The respiratory activity per unit of body weight, being proportional to the ratio of surface to mass, would of course vary but little in the goats, and would only be about a fourth greater in a goat of 15 kilos than in one of 30 kilos.

to their susceptibility but by the bands into which they had been marshalled for the pressure experiments. A factor of considerable importance, which is to a large extent beyond control, is the activity of the goats at the moment. Some goats are naturally vivacious while others are almost constantly lethargic. These individual idiosyncrasies are no doubt of some moment in relation to susceptibility, but the customary habits of a group of animals may be altogether upset by an incompatible companionship in the chamber during an experiment.

One group of measurements gave for example the results shown in the following table. The CO₂ production of each group was determined on four separate occasions under conditions similar to those obtaining in the pressure experiments.

TABLE XX.

Females.						Males.					
No. of goat	Weight kilos	Expts.	Ill-nesses	Pressure lbs.	CO ₂ gms. per kilo per hour	No. of goat	Weight kilos.	Expts.	Ill-nesses	Pressure lbs.	CO ₂ gms. per kilo per hour
XII A	14.2	14	4	0 45 45 45	0.921	3	19.2	15	3	0 45 25 45	0.669 0.697 0.959 0.967
XV A	15.5	17	8		1.104	4	20.0	16	1		
XVIII A	31.4	17	2		1.020	X A	17.8	17	6		
XXI A	17.0	17	11		0.852	XI A	23.0	17	2		
XXIII A	22.0	13	0			XVI A	22.6	16	4		
						XIX A	25.3	17	7		
Average	20.0	78	25 (32 %)	—	0.974		21.3	98	23 (23 %)	—	0.823

The average size in each group is about the same, and the sex incidence for all goats of Series III is the same. The results therefore appear to show that the males are 32 % less susceptible and produce 17 % less CO₂—a result which cannot be correlated with theory.

The only experiments made with the animals grouped according to their susceptibilities gave much more rational results. Great care was taken in this series to make the conditions as nearly identical as possible in all four observations; the animals were kept in the dark and remained quite quiet throughout. The results show a CO₂ production by the susceptible animals one-sixth less than that of the non-susceptible.

Influence of blood volume. The volume of the blood was determined in 8 goats, in 7 of which the susceptibility to caisson symptoms had been ascertained. The method used was the simple one of Welcker, in which, after taking a standard sample of arterial blood, the animal is bled to death and then thoroughly washed out with salt solution. The

TABLE XXI.

Animals							Respiration					
No. of goat	Sex	Weight	Total		Selected		No. of expt.	Temp.	Bar. m.m.	Duration in hours	CO ₂ gms. per hour	
			Decomp. ¹	Ill	Decomp.	Ill					per kilo	per 1000 sq. cms.
4	M	18·6	15	0	10	0						
XIA	M	20·8	16	1	11	1	I	12°	776	5	0·717	1·853
XVIII A	F	30·7	14	0	10	0	III	14°	775	5	0·751	1·941
XXIII A	F	25·2	12	0	8	0						
Average		23·8	57	1 (2%)	39	(2½%)					0·734	1·897
<i>Susceptible goats.</i>												
XVA	F	15·0	15	6	10	4						
XXI A	F	15·4	15	8	10	6	II	14°	775	5	0·624	1·501
XIX A	M	21·3	15	7	10	5	IV	13°	766	5	0·704	1·693
XIII A	F	25·2	16	5	11	2						
Average		19·2	61	26 (43%)	41	17 (41%)					0·664	1·597

¹ These figures are given up to the date at which the respiration experiments were made. Some time elapsed before the final susceptibilities were ascertained: these were 8% for the non-susceptible group and 45% for the susceptible animals.

tissues were not afterwards extracted with water: the red colouring matter so obtained is so small in amount that it can have little influence on the final result, and Douglas¹ has shown that additional difficulties are thereby introduced. For purposes of calculation the specific gravity of the blood has been taken as 1050. The results and the decompression records of the goats are given in the two following tables. The figures should be read in relation to the "clean" weight, *i.e.* the crude weight less the weight of the contents of the alimentary canal, which in these animals is very considerable.

The results seem to indicate that there are two types of blood volume in goats: one about 7½% of the clean body weight and the other about 6½%, the first type being also associated with a higher percentage of haemoglobin. No relation between blood volume and susceptibility is apparent; thus goats 2 and XIII, both males, have identical blood volumes and differ about as widely in their susceptibility as any two goats which have come under our notice.

Conclusions. Of the four factors considered in detail, it appears therefore that age, sex and blood volume were without appreciable influence. Pregnancy and a low rate of respiratory exchange seem to favour the occurrence of symptoms.

¹ *Journal of Physiology*, vol. xxxiii. (1906), p. 499.

TABLE XXII.

No. of goat	Sex	Whole weight kilos	Weight of contents of stomach and intestines		Volume of blood c.c.	Mass of blood per cent. of		Haemo- globin p.c. of human standard ¹
			Total kg.	Per cent. of whole weight		Whole weight	Clean weight	
1	M	19.9	3.8	19.1	1006	5.31	6.56	74
XIII	M	18.9	4.3	22.7	883	4.91	6.36	—
2	M	18.0	3.7	20.5	874	5.10	6.42	64
X	M	17.0	3.0	17.6	833	5.14	6.25	72
XVI	M	11.3	2.3	20.3	592	5.50	6.91	64
A	F	31.2	5.2	16.7	1874	6.31	7.57	78
XVIII	F	27.1	4.6	17.0	1395	5.41	6.47	75
XVII	F	24.4	4.0	16.4	1520	6.54	7.83	84
Average		21.0	3.9	18.8	1122	5.53	6.80	73
Average of males		17.0	3.4	20.0	838	5.19	6.50	68.5
Average of females		27.6	4.6	16.7	1596	6.09	7.29	79

¹ The red blood corpuscles of goats are very small, about 4μ in diameter (Jolly, *C. R. Soc. de Biol.*, vol. LXIII (1907), p. 210).

TABLE XXIII.

Pressure 75 lbs.

Exposure minutes	Decomp. minutes	Goat 1	2	X	XIII	XVI	XVII	XVIII
15	30 un.	0	slight bends	0	0	bends	0	slight bends
15	30 un.	0	para- plegia	0	0	0	0	0
15	30 st.	0	0	0	0	0	0	0
15	30 st.	bad bends	bends	0	0	0	0	0
30	30 st.	bends	temp. paral.	bends	0	bends	bends	0
60	30 st.	bends	0	0	bends	0	dyspnoea	0
120	70 un.	bends	para- plegia	0	0	bends	temp. paral.	0
120	70 st.	0	bends	bends	0	0	0	0
15	1	—	—	—	—	bends	—	—
15	10 un.	—	—	bad bends	0	bends	—	—
30	30 un.	—	—	—	—	bends	—	bends
30	30 st.	0	—	0	0	temp. paral.	—	bends
60	30 st.	0	—	0	0	0	—	obscure
120	30 st.	bad bends	—	bad bends	bends	bends	—	bad bends
240	30 st.	—	bad bends	bends	0	temp. paral.	—	bends
240	30 un.	—	—	temp. paral.	—	—	—	—

But there are doubtless other particulars which, alone or in combination, are of fundamental importance. Such other factors we have not yet been able to examine in detail. Fatness for example can be gauged only in the dead, and, though we have the distinct impression that the goats which die easily (*i.e.* under circumstances of pressure, exposure and decompression which cause very few severe symptoms and deaths) are fatter than those which are killed with difficulty, we have had no means of extending our observations on this head to the great majority of our animals¹. Fatness also involves a low rate of respiratory exchange per unit body weight. There are grounds in human experience for holding that age may have an important share in the production of symptoms and Hill and Greenwood have recently shown² clearly that young animals (rats, rabbits and cats) are far less susceptible than adults of the same species. All the goats used by us appeared to be adult; in the two cases (XIII A, XXXII A) in which old age had obviously set in, the susceptibility seemed to be somewhat above the average, but the ages of the animals as a whole were unknown. In any case such a factor as old age must be reduced to simpler components before it can be correlated with the theory of decompression.

8. *The pathology of caisson disease in goats.*

We have hitherto dealt exclusively with the symptoms exhibited by the experimental animals rather than with the actual or possible presence of bubbles within them. We have however made a number of observations on the post-mortem appearances of goats after decompression, which may be shortly dealt with here. Most of the animals had died from caisson disease but in other instances they were killed at varying periods after decompression.

The presence of bubbles *in vivo* must be inferred from their discovery post-mortem with considerable caution. The supersaturation of the body may be such that the separation of the gas as bubbles may take place after death. There are reasons for supposing that the living body presents nothing in the way of points or surfaces on which bubbles might arise in the blood and tissues as they do upon the glass and dust in soda-water, and a remote analogy may perhaps be drawn

¹ We have since examined this point by direct analysis of rats and guinea-pigs divided into susceptible and non-susceptible groups by decompression experiments. The results, which will be published in detail later, show that fatness is a very important factor in individual susceptibility to death.

² Meeting of Physiological Society, Nov. 1906.

with the relations of the vessels to the separation of fibrin *intra vitam*. Death may well alter this condition in some degree, and in any case the time factor is of importance as well as the foreign manipulation which examination involves. During life no portion of the blood remains in a supersaturated state for more than the time required for it to return from the tissues to the lungs. We have already seen that the duration of this period is probably of very great importance as regards risk of bubble formation. On cessation of the circulation the blood remains in a supersaturated state for an indefinite period. In one instance at least we have actually observed such post-mortem separation of bubbles: a rabbit was killed immediately after 75 lbs., 2 hours, 31 minutes stages, opened up at once and no bubbles found¹; an hour later a few bubbles were found in the inferior *vena cava*. In another case bubbles in the bladder were seen to increase considerably in number and volume during the progress of the examination.

The possibility of air being introduced from without into the veins must also be considered. Ewald and Kobert showed that air might be forced into the pulmonary capillaries by an increase of intra-pulmonary pressure such as may occur in severe dyspnoea. We have seen bubbles in large quantities in the meningeal veins, and in small numbers in the superficial veins of a fore-foot, under circumstances which left no reasonable doubt that they had been sucked into the vessels during the somewhat violent manipulations used in opening the skull and skinning the leg respectively.

There is not much doubt that some of our animals which showed no symptoms must have had bubbles present in the blood. Catsaras decompressed a dog in 1 minute after 2 hours exposure at 65 lbs.: it showed no symptoms and was killed 6 hours later, when fine bubbles were found in the blood. Heller, Mager and von Schrötter (pp. 790, 882) record two dogs which were killed 10 minutes after sudden decompression after 65 and 52 minutes at 15 and 18 lbs. respectively: in both cases bubbles were found in the heart², though there is abundant evidence to show that dogs never show any symptoms after decompression from such low pressures. In our own animals attempts were made to see bubbles in the retinal vessels during life. Though an

¹ The vessels and bladder in the rabbit are so thin-walled that bubbles can be seen with certainty if they are present.

² On the other hand dogs (showing no symptoms) killed after sudden decompression after 16 minutes exposure at 2·8 atmospheres, 5+ at 3·5, 12 at 4·5 and 5 at 4·7, showed no bubbles in the blood: these were however found in three other dogs after 10+ minutes at 4·0 atmospheres, 16 and 72 at 4·5.

excellent view of the fundus may easily be obtained, no bubbles were ever seen even in animals with severe dyspnoea, so that the method cannot be taken as giving any indication of the absence of bubbles in the blood. Some of these animals died and plenty of free gas was found in the retinal vessels post-mortem. Four animals which showed no symptoms were killed within ten minutes after decompression with the following results:

TABLE XXIV.

Series and number of goat	Pressure lbs.	Exposure (actual) minutes	Decompression minutes	Bubbles in blood	Results of similar experiments in other goats			
					Number	Bends	Severe symptoms	Death
3: XXVI A	25	120	$\frac{1}{2}$	absent	23	2	0	0
1: II	45	26	6	absent	15	3	0	0
2: XIV	75	19	31 stages	present	29	5	0	0
1: 1	78	30	9	absent	4	0	0	1

These goats were killed before the expiration of the appropriate period for the development of bends. The experience of similar experiments indicates that they might have shown symptoms if they had not been killed. Yet three out of four had no bubbles in the blood. A few observations on rabbits on the other hand gave rather different indications. Seven rabbits in seven different experiments were exposed to 75 lbs. for periods of from 15 to 120 minutes and killed immediately after decompression in 31 minutes by stages. There is no question but that it would be the very rarest occurrence for a rabbit to have any symptoms under these circumstances, but in four of the animals we found bubbles in the heart or great veins. These may however have been formed post-mortem, and in one such case they were observed to appear some time after death.

The post-mortem appearances observed may now be shortly described. It should be remembered that most of the animals dealt with here died under circumstances of experiment less severe than those of other observers. The pressure was in almost all cases 75 lbs. and in a majority of instances the decompression was not instantaneous. These considerations probably afford the explanation of the somewhat less emphatic changes which we have noted. The naked eye appearances were in nearly every case supplemented by microscopical examination of many of the organs.

Lungs. The amount of blood in the lungs depends upon the condition of the heart: in severe cases, with the pulmonary artery choked with bubbles and the right heart distended, they are pale and bloodless; in other instances the quantity of blood appears about normal.

Haemorrhages may occur and small blood clots are not infrequently found in the trachea. A very marked, scattered, lobular emphysema is almost constant; the only explanation appears to be that some bronchioles are more or less impervious during decompression. Examination of fresh material showed that bubbles may burst out of the capillaries into the interstitial tissue of the lungs, and presumably therefore also into the alveoli. The same was found in rabbits which were killed by the injection of small quantities of air into the veins. Nothing resembling the exudative process seen in oxygen poisoning was ever found in animals exposed to simple air pressures.

In all fatal cases (with one exception) more or less abundant bubbles were found in the blood. In severe, rapidly fatal cases, the right heart is much distended with bubbles, the pulmonary vessels plugged with froth and the left heart nearly empty. The block in the pulmonary artery may indeed be so complete that the left auricle is collapsed and puckered up. In other animals, which have lived for 20, 30 or 60 minutes or longer, the two sides of the heart are equally full of blood and the right heart is not distended. The immediate cause of death, in all but three cases, which died many hours after decompression, was clearly pulmonary air embolism, and this is doubtless the cause of the urgent dyspnoea already noted. In two cases death ensued without any dyspnoea. Both these animals were intentionally killed by very severe experiments, viz. 75 lbs. for 1 hour (goat XXI) and 3 hours (XVIII A) with decompression in $1\frac{3}{4}$ and 1 minutes. Both showed no symptoms for 5 minutes and collapsed and died quietly in 10 and 12 minutes respectively. Such animals must be regarded as being so overwhelmed by sudden asphyxiation that they exhibit only the symptoms of deficiency of oxygen and not those of the accumulation of carbon dioxide.

The fatal case in which no bubbles were found in the blood post-mortem was in goat XXVII A. After 75 lbs., 15 minutes, 42 seconds it showed pain, ate part of a note-book, and became paraplegic. It was found dead next morning¹. In several other cases of delayed death, and in one (XVI A) in which the animal died in 3 hours², the quantity of bubbles found seemed to be altogether inadequate to produce a fatal result. One may suppose that they had been previously more

¹ Heller, Mager and v. Schrötter (p. 852) record a case in a dog fatal in 6 hours after decompression in which no free gas was found in the vessels.

² This animal had however been recompressed and died under a state of recompression: see protocol e, Appendix III.

numerous and that oxygen starvation resulted in death at a time when the aeration of the blood had been restored. This form of delayed death from deficiency of oxygen is well-known in *e.g.* carbon monoxide poisoning. It might be for instance that temporary obstruction of the coronary arteries or portal capillaries caused fatal degenerative changes in the heart muscle or liver cells.

The distribution of the bubbles in the different parts of the vascular system shows several peculiarities. If only a few are present, all may be collected in the smaller branches of the pulmonary artery with none in the right heart. The left heart generally contains a few bubbles; the amount there and in the arteries roughly varies inversely with the rapidity of death unless decompression has been very quick. Smallness in the amount of bubbling affords the heart the best chance of being able to pass on the froth to the arteries, and cases which die slowly seem to show distinctly more arterial bubbles than those which expire almost at once. The veins contain variable quantities of bubbles, but always more than the arteries. They are especially abundant in the mammary, mesenteric, spermatic and portal veins, coronary vessels, and notably few in the veins on the surface of the stomach and in those of the brain and spinal cord. In several instances we have noticed great accumulations of froth in the liver while the spleen at the same time showed no bubbles in the blood flowing out on section. This massive portal embolism is probably the cause of the multiple small capillary haemorrhages which are frequently seen in the omentum and mesentery. Blocking of the portal circulation might also give rise to general symptoms of a very serious character.

It should be noted that the liver is particularly badly situated for getting rid of excess gas during and after decompression; nearly all the blood reaching it is already partly saturated by passing through the intestines, &c. The liver also contains much fat.

Lymph. The lymph in the thoracic duct has been noted to be full of froth on several occasions.

Other liquid areas. Bubbles have very seldom been found in the *aqueous humour*; the blood supply is considerable, so that their absence is probably to be attributed to the excess gas being carried off during decompression and the period which the supersaturation phenomenon adds, for practical purposes, to the actual time occupied in reducing the pressure to normal. The *vitreous humour*, on the other hand, has a poor blood supply and its consistence is such that any bubbles forming there would remain *in situ*. On only one occasion have bubbles been found

(goat XXVI, 75 lbs., 2 hours, 31 minutes stages), when they were seen in a layer close against the ciliary body. Their absence is explicable on the ground that the vitreous humour would take a very long time to saturate. The *bile* often contains bubbles: they were noted in one goat exposed at 75 lbs. for 15 minutes and killed immediately after decompression in 31 minutes stages, and in 8 animals exposed to the same pressure for 1—4 hours, but not in two animals exposed for 15 minutes which died 30 minutes after decompression in 10 minutes and 30 minutes uniformly respectively. The *urine* found in the bladder post-mortem is remarkably free from bubbles; on two occasions only has free gas been found. We have evidence here that the phenomenon must be due to supersaturation and the absence of "points," since we have very frequently observed goats pass urine after decompression which frothed freely on coming into contact with foreign surfaces. This is often seen in animals which show no symptoms. Thus in one experiment, seven goats were exposed at 45 lbs. for one hour and decompressed in 30 seconds. One had bends 19 minutes later. Within 24 minutes after decompression four animals passed urine; in two cases this frothed up freely as it ran over the pavement while in the other two (including the goat which had bends) no bubbles could be observed. It is somewhat striking to observe the transparent bladder of the rabbit containing urine quite free from bubbles while the vesical veins coursing over its surface are full of froth. The *cerebro-spinal fluid* rarely shows any bubbles: they have been seen only three times, in all cases after long exposures (1 to 3 hours) at 75 and 51 lbs. with sudden decompression. *Synovial fluid* is almost always full of bubbles; exposure for 15 minutes at 75 lbs. is sufficient to cause their presence, while decompression in 100 minutes uniformly is not enough to prevent their formation. In animals which have died within 3 hours of decompression, we have found them in every case. *Amniotic fluid* is dealt with below. Bubbles have been seen after very severe experiments in the *pericardial* and *peritoneal fluids* when present, and in the serous contents of a mammary cyst, but not in the milk. We have not seen any accumulations of gas in the serous cavities.

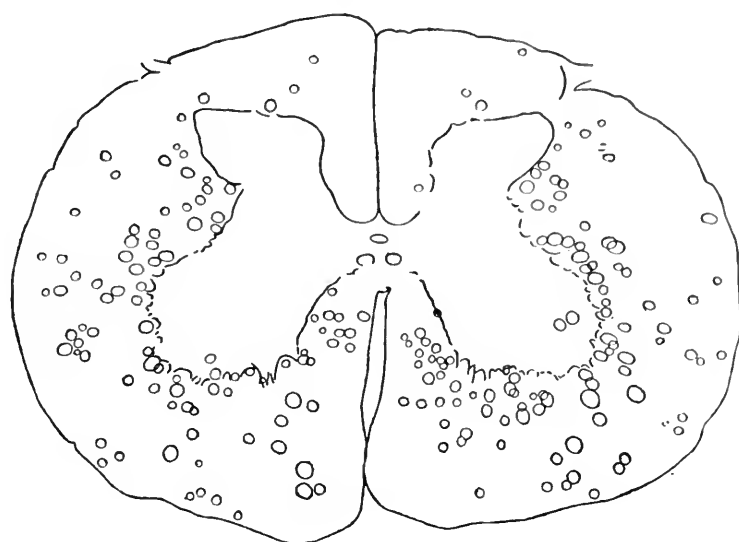
Solid organs. Fat commonly shows bubbles, often in extreme abundance. They are more numerous in the abdominal than in the subcutaneous fat; the latter is much more vascular. Other solid organs for the most part show no bubbles outside the blood vessels; a few are sometimes found in the liver and the spinal cord may contain large numbers. In the liver it is very difficult to determine whether any

bubble is inside a capillary or not, and we have failed to find clear evidence of bubbles outside blood-vessels, still less actually within tissue cells, in cardiac or skeletal muscle, spleen, kidney, suprarenal, salivary glands, thymus, thyroid and parathyroid, pancreas, lymphatic glands, haemolymph glands, nerves, posterior root ganglia, testis, ovary or mammary gland, though an extensive systematic histological examination has been made of more than 20 goats exposed at 75 or 45 lbs. for from 10 minutes to 4 hours and decompressed in from 30 seconds to 100 minutes.

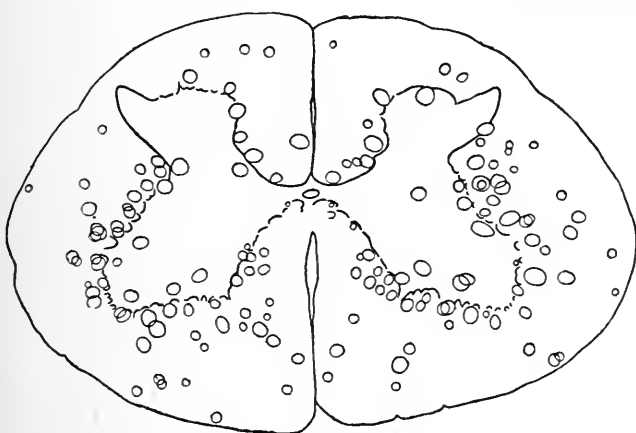
This condition has no very obvious explanation. It is curious, for instance, to see the spermatic vein (and sometimes artery as well) full of froth and yet find no evidence of bubbles in the tissues which it drains: the same thing is also shown most strikingly in the mammary gland and vessels. There can be no doubt that these tissues must be fairly completely saturated in 4 hours and it is impossible that the excess should be removed from the tissues more quickly than from the blood. It follows that the blood must stand in an unfortunate relation towards bubbling in that it effervesces with a smaller difference of pressure, within and without, or with the same difference of pressure in a shorter time, than do the more solid organs. It seems unlikely that this difference depends on the motion of the blood. Rhythmical pulsating circulation through a smooth elastic system can hardly function as a shaking which would be efficient in bringing out free gas. Even if it did, it is not easy to see why the tissues are not affected by the pulsations in the same way, though perhaps not to the same degree, since isolated collections of fluid, as in the joints, may bubble very easily. One can only suppose that the dissolved particles of gas find in the tissues obstacles, visible and invisible, more obstructive to their aggregation into bubbles than those occurring in the blood.

Bubbles once formed in the blood will also increase in size more readily, since their movement will continuously keep them in contact with fresh portions of supersaturated liquid.

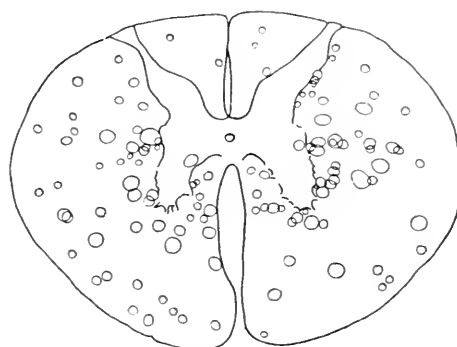
Among the solid organs, bubbles outside the vessels are found most frequently in the central nervous system. The fatty nature of this tissue is probably important in this respect. The brain is singularly free, both by direct examination and by the study of secondary degenerations. The cord may however contain numerous bubbles, and a study of their occurrence and distribution gives interesting results. In the first place they may occur in areas of softening after comparatively mild experiments (*e.g.* 45 lbs., 2 hours, 10 minutes): in this case



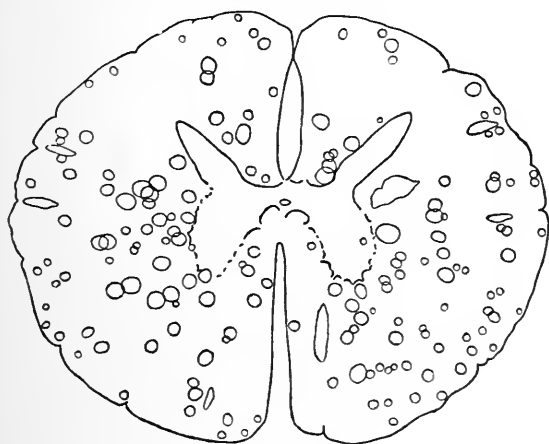
6th cervical.



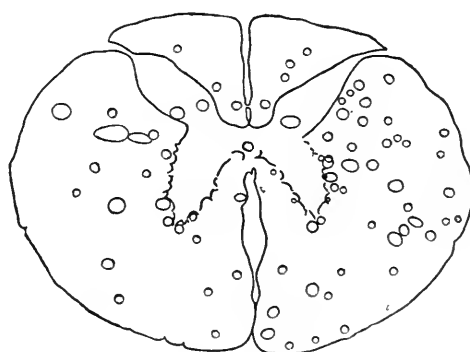
4th lumbar.



11th dorsal.



2nd cervical.



3rd dorsal.

Fig. 7. Shows the distribution of extravascular bubbles in five regions of the spinal cord of goat 3 (series IV). The animal died of oxygen poisoning soon after the beginning of a decompression of 133 minutes duration by stages after 3 hours exposure at 81 lbs. in an atmosphere containing 36% oxygen. The bubbles are practically confined to the white matter and are there especially concentrated in the boundary zone where the circulation is least good. Each diagram is a composite drawing showing all the bubbles in 0.4 mm. length of cord.

they are confined to the area in which the circulation has been brought to a sudden standstill by a collection of gas. On the other hand they may be found in the cords of animals which have died immediately after a drastic decompression. This is however rather exceptional. Thus the cords of three animals decompressed in less than a minute after 1 hour at 100 lbs. and 1 and 2 hours at 75 lbs. contained numerous bubbles, while in two animals treated in the same way after exposure at 75 lbs. for 1 and 3 hours respectively, none were found.

The distribution of the bubbles when numerous is in harmony with the theoretical conclusions derived from the blood supply. They are for instance least numerous in those segments with an abundant blood supply (lumbar enlargement), and are almost confined to the white matter, those found in the grey substance being distributed along its periphery towards the boundary zone between the superficial and deep vessels. Thus one cord (goat XXI: 75 lbs., 1 hour, $1\frac{3}{4}$ minutes) contained the following bubbles in 412 cubic millimetres in different parts:

Segment	Grey matter	Posterior columns	Ant.-lat. columns	White matter	Total
2nd cervical	14	141	215	197	175
5th dorsal	11	23	95	87	79
1st lumbar	0	32	161	140	140
4th lumbar	2	15	37	28	19
Average	7	53	127	113	103

Fig. 7 shows the distribution of bubbles in another case: note the paucity of bubbles in the grey matter and their concentration in the boundary zone.

The distribution of the areas of softening is also important. With one exception, these are most marked in, and usually confined to, the lower dorsal and upper lumbar segments where the blood supply of the cord may on many grounds be surmised to be at its minimum. They affect only the white matter. Now the only parts of the body in which we have found appearances resembling embolic infarction are the white matter of the spinal cord and the fat. The latter has on several occasions been found to contain large and small areas of necrosis. We have obtained no evidence of infarction of the spleen, kidney, heart-muscle, &c. The distribution of small bubbles by the arterial blood stream must be universal. They probably lodge in many places: while they are rapidly pushed forward in the grey matter and in most other tissues, if they lodge among the fatty surroundings of the capillaries of the white matter, or in actual fat, they quickly increase in size to such

an extent that their removal becomes impossible. It is also clear that in consequence of the slow circulation in the white matter, and especially in such inactive parts as the lower dorsal segments, bubbles have plenty of time to increase in size in the circulating blood. The condition of supersaturation will also last much longer in the white than in the grey matter. The cause then of these areas of softening in the cord is not ordinary embolism, but embolism which becomes effective to produce infarction by reason of the effect on the size of the embolus of the local conditions of the circulation rather than from any of those peculiarities in the resistance of the different tissues to lack of oxygen, or in the freedom of collateral circulation, which determine the topography of common infarcts.

The presence of bubbles in the uterine contents. We may group together here a number of casual observations which have been made on the distribution of bubbles in the foetus and amniotic fluid of pregnant goats dying of caisson disease. The pressure was in all cases 75 lbs.

TABLE XXV.

Number of goat	Exposure minutes	Decompression minutes	Time of death after decompression minutes	Bubbles present in the			Development of foetus
				maternal blood	foetus	amniotic fluid	
XXIII	15	31 stages	30	+	0	0	advanced
XXII	120	31 „	75	+	0	+	„
XX	240	31 „	40	+	live + dead 0	+	6 inch 4 inch
XXI	60	1 $\frac{3}{4}$	10	+	+	+	advanced
XI	240	31 uniform	25	+	0	0	1 inch
XIV	15	31 stages	killed at once	+	0	0	$\frac{1}{2}$ inch
XVIII A	180	1	12	+	+	+	advanced
XXVIII A	180	4 $\frac{1}{4}$	24	+	0	0	$\frac{1}{2}$ inch
XXXII A	180	4	27	+	0	0	4 inch (dead)

These observations seem to be fairly concordant. In 15 minutes the uterine contents have not taken up much excess of gas (XXIII), nor does a dead foetus absorb any (XX, XXXII A). In one hour both foetus and amniotic fluid have taken up abundant excess (XXI), which may, if death be long delayed after a rather slow decompression, be discharged from the foetus more quickly than from the amniotic fluid (XXII)¹. With a very young foetus, the circulation is probably too

¹ Two pregnant guinea-pigs were exposed for 1 hour at +100 lbs. and decompressed in 34 minutes by stages: they showed no symptoms. On being killed 5 hours later, numerous bubbles were found in the amniotic fluid but nowhere else.

active and the bulk of fluid too small to favour bubbling (XI, XIV, XXVIII A).

The amniotic fluid, which contained in this case only a faint trace of proteid, may show the phenomenon of supersaturation to an exquisite degree. In goat XXI it was especially noted that a large bubble was present in the amniotic fluid on removing the uterus from the body. After free shaking to bring out any more gas, the uterus was opened and the contents poured into a glass vessel. Contact with this foreign surface immediately produced a great froth of fine bubbles.

The free gas runs together into one large bubble. Advantage was taken of this convenient circumstance in two instances to make analyses. The samples were collected over water and in XXII analysed at once; in XX they were kept for 20 hours over water before examination and in this case therefore the figures for CO₂ represent minimal and those for oxygen maximal values.

	XXII	XX Live foetus	XX Dead foetus
Total gas c.c.	16	27	10.5
CO ₂ per cent.	16.23	3.55	2.73
O ₂ ,,	1.10	2.14	0.85
N ₂ ,,	81.90	94.14	96.21
Combustible gas (calculated as CH ₄ and H ₂)	0.77	0.17	0.21

These results correspond with those of Bert (pp. 955, 961) of the free gas in the heart: they are not in accord with those of Heller, Mager and von Schrötter (p. 800) who found 15.31 and 7.18 per cent. of oxygen in the free gas collected from the hearts of dogs. It is somewhat significant that if this excess of oxygen is calculated as an air leak, the figures of Schrötter correspond exactly with those of Bert and our analysis XXII.

Duration of bubbles. It is difficult to say how long bubbles may remain in the vessels and tissues after their first formation in animals which survive¹. The question is much complicated by the fact that we have reason to believe that bubbles may continue to form for a long, and quite unknown, time after decompression. This is probably especially marked in cases in which either local blocking of the blood supply has occurred, or the circulation has been slowed generally by a greater or less degree of cardiac and pulmonary obstruction. It would

¹ Zografidi (*Revue de Médecine*, 1907, p. 159) records the finding of numerous bubbles in the peripheral vessels, but not in the heart, of a diver who was paralysed and died 33 days after decompression !

appear likely that bubbles once lodged in the lungs would probably stop there for a considerable time, since their gaseous composition would quickly approximate to that of the alveolar air and there would be no considerable difference of tension to encourage their removal. Such results as we have which bear on the matter are collected in the next table. It will be seen that bubbles have been found in the blood of one animal which died two days after decompression (and that in an animal which had shown no dyspnoea) and in the joints up to 26 hours. In the substance of the spinal cord bubbles may persist far longer: in two cases we have found them 15 days after the last exposure to pressure and in one 27 days after the last occurrence of symptoms.

TABLE XXVI.

Goat	Pressure lbs.	Exposure minutes	Decompression minutes	Result hours	Bubbles present in	
					Blood	Joints
XXII (Series I)	75	35	40 stages	died 39	+	—
XXV A	75	120	100 uniform	„ 16½	+	0
XXVII A	75	15	$\frac{4}{8}$	„ 15*	0	0
XV A	45	120	$\frac{1}{2}$	killed 24	0	0
4	45	120	$\frac{1}{2}$	„ 26	0	+
XIII A	45	120	10 uniform	„ 72	0	0
XA	75	10	$\frac{5}{8}$	„ 96	0	0
XXIII A	45 to — 6	180	6	„ 144	0	0

* Found dead next morning.

A histological point of some practical importance arises in connection with the size of the bubbles found in the blood. The bubbles soon run together into large bullae after death so that it is necessary to make the examinations immediately after death in order to observe approximately the true state of affairs. It will then be found that there are no bubbles so small as to be of strictly microscopic dimensions. Nor are any very small bubbles found in the spinal cord; in any one case all the bubbles are about the same diameter, commonly some 25 microns. The same is true of the bubbles given off on decompressing water, salt solutions, serum, blood, and even such thick solutions as gelatine or agar. At the same time however it is possible to produce bubbles which are truly microscopic and which last some hours in some sticky solutions such as gum and treacle. The energy required to aggregate particles of dissolved gas into a bubble is evidently considerable, and there is the same difficulty in the formation of free gas bubbles from solution in liquids as there is in the separation of liquid particles from solution in gases and of solid particles from solution in liquids.

Extremely minute bubbles are unstable in the same way as extremely minute droplets of water condensing from supersaturated air, or salt crystals forming in a supersaturated solution in water: in all cases the tendency is to reduce the free energy (surface tension) by reducing the ratio of surface to mass, and accordingly the smallest bubbles, droplets or crystals as the case may be, are rapidly, in the case of bubbles practically instantaneously, abolished to produce macroscopic masses. This is well seen on watching under the microscope a stream of bubbles coming off some "point" in soda water. It follows that if the concentration of dissolved molecules of gas is not higher than some unknown point, bubbles will not be formed. It is possible that the absence of bubbles from most of the solid tissues is to be explained by this non-existence of very small bubbles and the mechanical difficulties of the rapid aggregation of a sufficient number of molecules to produce large bubbles. It is also doubtless connected with the period of delay in bubble formation whereby an animal, for practical purposes, gains several minutes over the actual time of decompression.

It is reasonable to suppose that the temporary paralyses are due to temporary ischaemia from air bubbles in the vessels. The more lasting palsies are undoubtedly caused by obstruction sufficiently complete to produce softening and necrosis. As already mentioned, the change is confined to the white matter and in nearly all instances affects only the lower dorsal or upper lumbar region. In these segments the bulk of cord destroyed may be very extensive: thus in goat XXIII A fully three-quarters of the lateral columns were destroyed from the eighth dorsal to the second lumbar segment over a length of rather more than five inches, and in goat XV A the softening involved nearly the whole of the lateral columns and parts of the anterior and posterior columns for a length of four and a half inches. Such cases may recover to a remarkable degree, and eventually show objective signs of paralysis so slight as to be hardly perceptible except to those familiar with the individual animals.

The only other tissue in which we have found any signs of the results of circulatory obstructions is the intra-abdominal fat. Large masses of necrosed fat have been occasionally met with, especially in the fat lying below the kidneys. Only late stages have been seen: the necrosed areas are then surrounded by a well-marked ring of giant-cell reaction, and the surface layers are mostly converted into a calcium soap.

No evidence of infarction in other organs has been seen: the rarity

of such changes seems to afford pretty good evidence that the duration of any obstruction in such organs as the spleen or kidney cannot be very long.

The pathological changes underlying the chief symptoms have been already sufficiently noticed except as regards bends. The exact cause of bends is not known. They have been attributed to bubbling in the central nervous system, chiefly on the ground that human experience shows that they are very frequently bilaterally symmetrical. This fact however cannot be taken as indicating any such origin in view of the complete symmetry of the limbs (where the symptoms occur) and the uniform symmetry of the causative agent throughout the body. In two animals which were killed soon after decompression when they showed bends only, we could find nothing abnormal in the cord, posterior root ganglia, or nerves, and there is abundant evidence from a number of goats that the cause of bends does not produce such lesions in the nervous system as are followed by secondary degeneration which can be revealed by the methods of Marchi or Weigert. The two following goats may be cited in detail as to this point: in neither was any degeneration found. Goat XXI (Series II) was used in seven experiments between November 26th and January 18th: it had bends on December 5th, 11th and 18th (the last being noted as "bad bends"), and dyspnoea, nearly fatal, on January 2nd: it was killed on January 18th. Goat XV A (Series III) was exposed 27 times between February 2nd and June 10th: it had bends on February 2nd, 20th, 22nd, March 3rd, 5th, May 13th, 15th, 27th and June 6th: it was killed on June 11th. Thorough examination of the pons, medulla and cord showed no secondary degeneration in either animal. There are therefore reasons for thinking that the cause of bends is peripheral rather than central. The constant presence of bubbles in the joints has been already mentioned, and they seem to afford a fairly probable explanation of most of the cases. Even in those cases in which the muscles are the seat of pain, it is quite possible that a sensation actually originating in or around the joints is referred to other parts. The joint pains in man are often relieved by flexion, and goats evidently try to obtain ease in the same way (see Plate VI). This fact adds strong confirmation to the conclusion that the origin of the pain is in or about the joints.

We have already seen that bends, while not the first symptom to appear as the duration of exposure to high pressure is increased, are the last symptom to disappear as the decompression is extended, that bends in short arise in parts of the body which saturate and desaturate

rather slowly. The synovial fluid satisfies this criterion; on the other hand the tendons and other dense tissues about the joints are not in disagreement with it.

Bends occur with a lower degree of supersaturation with air than any other symptom of compressed-air illness. In goats they are readily produced after exposures to 30 lbs. or less. The fact that only a moderate degree of supersaturation is needed to produce them seems to explain the fact that although they are not the first symptom to appear as the duration of exposure to very high pressure is increased, yet a moderate duration of exposure suffices to produce them, in spite of the fact that they occur in parts of the body with a slow rate of circulation, as shown by the fact that they are the last symptom to disappear as the duration of decompression is prolonged.

SUMMARY.

1. The time in which an animal or man exposed to compressed air becomes saturated with nitrogen varies in different parts of the body from a few minutes to several hours. The progress of saturation follows in general the line of a logarithmic curve and is approximately complete in about five hours in man and in a goat in about three hours.

2. The curve of desaturation after decompression is the same as that of saturation, provided no bubbles have formed.

3. Those parts of the body which saturate and desaturate slowly are of great importance in reference to the production of symptoms after decompression.

4. No symptoms are produced by rapid decompression from an excess pressure of 15 pounds, or a little more, to atmospheric pressure, *i.e.* from two atmospheres absolute to one. In the same way it is safe to quickly reduce the absolute pressure to one-half in any part of the pressure scale up to at least about seven atmospheres: *e.g.* from six atmospheres (75 pounds in excess) to three (30 pounds), or from four atmospheres to two.

5. Decompression is not safe if the pressure of nitrogen inside the body becomes much more than twice that of the atmospheric nitrogen.

6. In decompressing men or animals from high pressures the first part should consist in rapidly halving the absolute pressure: subsequently the rate of decompression must become slower and slower, so that the nitrogen pressure in no part of the body ever becomes more

than about twice that of the air. A safe rate of decompression can be calculated with considerable accuracy.

7. Uniform decompression has to be extremely slow to attain the same results. It fails because it increases the duration of exposure to high pressure (a great disadvantage in diving work), and makes no use of the possibility of using a considerable difference in the partial pressure of nitrogen within and without the body to hasten the desaturation of the tissues. It is needlessly slow at the beginning and usually dangerously quick near the end.

8. Decompression of men fully saturated at very high pressures must in any case be of very long duration: and to avoid these long decompressions the time of exposure to such pressures must be strictly limited. Tables are given indicating the appropriate mode and duration of decompression after various periods of exposure at pressures up to 90 pounds in excess of atmospheric pressure.

9. Numerous experiments on goats and men are detailed in proof of these principles.

10. The susceptibility of different animals to compressed-air illness increases in general with their size owing to the corresponding diminution in their rates of circulation.

11. The average respiratory exchange of goats is about two-thirds more than that of man; they produce about 0.8 gram. of CO_2 per hour per kilogramme of body weight.

12. The mass of the blood in goats is six and a half or seven and a half per cent. of the "clean" body weight.

13. The individual variation among goats in their susceptibility to caisson disease is very large. There is no evidence that this depends directly on sex, size or blood-volume: there is some evidence that fatness and activity of respiratory exchange are important factors.

14. Death is nearly always due to pulmonary air-embolism, and paralysis to blockage of vessels in the spinal cord by air. The cause of "bends" remains undetermined; there are reasons for supposing that in at least many cases they are due to bubbles in the synovial fluid of the joints.

15. In our experiments bubbles were found post-mortem most freely in the blood, fat and synovial fluid; they were not uncommon in the substance of the spinal cord, but otherwise were very rarely found in the solid tissues.

APPENDIX I.

Details of the experiments made on Lieutenant Damant and Mr A. Y. Catto, Gunner, R.N., in the pressure chamber at the Lister Institute.

These experiments were undertaken in July, 1906, as a preliminary to actual diving experiments in very deep water.

In the first three or four the decompression was controlled from inside the chamber; in the rest from outside. The subjects remained closed in the chamber for half an hour after each experiment, the engine being also kept running so that recompression could be at once begun if any serious symptom developed. In addition to the actual period of exposure to each pressure, we have noted the virtual period of exposure calculated on the assumption that about half the time occupied in compression must be added (see above, p. 362).

In view of the results with goats, the occurrence of decompression symptoms seemed probable in the more severe experiments. No symptoms were, however, observed, except considerable itching of the skin of the fore-arms where it was uncovered. In the compressed air the well-known alteration in the voice, and corresponding abnormal sensations about the lips and mouth, were very marked at pressures exceeding 60 or 70 lbs.

I. July 25th. Actual exposure to 39 lbs. for one hour. Virtual exposure 69 minutes, decompression in 24 minutes:

Compressed to	39 lbs. in 17 minutes.	
Waited at	39 „ for 60 „	
Decompressed to	9 „ in 7 „	} 24.
Waited at	9 „ for 5 „	
Decompressed to	4 „ in 1 „	
Waited at	4 „ for 9 „	
Decompressed to	0 „ in 2 „	

II. July 26th. Actual exposure to 50 lbs., 27 minutes. Virtual exposure, 39 minutes. Started at 10.37 a.m. Decompression in 34 minutes:

Compressed to	50 lbs. in 24 minutes.	
Waited at	50 „ for 27 „	
Decompressed to	17 „ in 4 „	
Waited at	17 „ for 6 „	
Decompressed to	13 „ in 1½ „	
Waited at	13 „ for 3½ „	
Decompressed to	9 „ in 2 „	
Waited at	9 „ for 3 „	
Decompressed to	4 „ in 2 „	
Waited at	4 „ for 8 „	
Decompressed to	0 „ in 4 „	

III. Same day, 3.3 p.m. Exposure to 55 lbs. for 19 minutes.
Virtual 33 minutes. Decompression in 31 minutes:

Compressed to	55 lbs. in 28 minutes.
Waited at	55 „ for 19 „
Decompressed to	17 „ in 4 „
Waited at	17 „ for 5 „
„ „	13 „ „ 5 „
„ „	9 „ „ 5 „
„ „	4 „ „ 10 „
Decompressed from	4 to 0 „ in 2 „

The time taken for decompressing from 17 to 13 lbs., &c., was counted as time at 13 lbs.

IV. July 27th, 10.29 a.m. Exposure to 60 lbs. for 20 minutes.
Virtual exposure 36 minutes. Decompression in $37\frac{1}{2}$ minutes:

Compressed to	60 lbs. in $30\frac{1}{2}$ minutes.
Waited at	60 „ for 20 „
Decompressed to	22 „ in 5 „
Waited at	22 „ for 5 „
Decompressed to	17 „ in 1 „
Waited at	17 „ for 4 „
Decompressed to	13 „ in $1\frac{1}{2}$ „
Waited at	13 „ for $3\frac{1}{2}$ „
Decompressed to	9 „ in 1 „
Waited at	9 „ for 4 „
Decompressed to	4 „ in $1\frac{1}{2}$ „
Waited at	4 „ for $8\frac{1}{2}$ „
Decompressed to	0 „ in $2\frac{1}{2}$ „

V. Same day, 3.37 p.m. Exposure to 67 lbs. for 18 minutes.
Virtual exposure 36 minutes. Decompression in 36 minutes:

Compressed to	67 lbs. in 36 minutes.
Waited at	67 „ for 18 „
Decompressed to	22 „ in 3 „
Waited at	22 „ for 5 „
Decompressed to	17 „ in 1 „
Waited at	17 „ for 4 „
Decompressed to	13 „ in 1 „
Waited at	13 „ for 4 „
Decompressed to	9 „ in 1 „
Waited at	9 „ for 4 „
Decompressed to	4 „ in $1\frac{1}{2}$ „
Waited at	4 „ for $8\frac{1}{2}$ „
Decompressed to	0 „ in 3 „

VI. July 30th, 10.57 a.m. Actual exposure at 74 lbs., 15 minutes.
Virtual exposure 35 minutes. Decompression in 42 minutes:

Compressed to	74 lbs. in 39 minutes.
Waited at	74 „ for 15 „
Decompressed to	26 „ in 4 „
Waited at	26 „ for 5 „
Decompressed to	22 „ in 1 „
Waited at	22 „ for 4 „
Decompressed to	17 „ in 1½ „
Waited at	17 „ for 3½ „
Decompressed to	13 „ in 1 „
Waited at	13 „ for 4 „
Decompressed to	9 „ in 1 „
Waited at	9 „ for 4 „
Decompressed to	4 „ in 1½ „
Waited at	4 „ for 8½ „
Decompressed to	0 „ in 3 „

VII. July 31st, 11.0 a.m. Actual exposure to 80 lbs. for 12 minutes.
Virtual exposure, 34 minutes. Decompression in 51 minutes:

Compressed to	80 lbs. in 44 minutes.
Waited at	80 „ for 12 „
Decompressed to	31 „ in 3 „
Waited at	31 „ for 5 „
Decompressed to	22 „ in 1 „
Waited at	22 „ for 4 „
Decompressed to	18 „ in 1 „
Waited at	18 „ for 4 „
Decompressed to	15 „ in 3 „
Waited at	15 „ for 2 „
Decompressed to	13 „ in 1 „
Waited at	13 „ for 4 „
Decompressed to	9 „ in 1 „
Waited at	9 „ for 9 „
Decompressed to	4 „ in 2 „
Waited at	4 „ for 8 „
Decompressed to	0 „ in 3 „

APPENDIX II¹.

A DIARY OF THE DEEP DIVING EXPERIMENTS CARRIED OUT OFF
ROTHESAY, ISLE OF BUTE, FROM H.M.S. *SPANKER*, AUGUST, 1906.

Monday, 20th August.

H.M.S. *Spanker* arrived at Rothesay about 7 p.m., and was met by Drs Haldane and Rees and Mr Catto, Gunner, R.N. Arrangements were made to commence experiments the following day.

Tuesday, 21st August.

All the pumps to be used in the experiments were tested up to a pressure of 200 feet, and the leakage at this pressure measured. The pressure gauges, which had been specially graduated for these experiments, were tested and found to give correct readings. The method of testing employed was to attach the free end of the diving hose to a lead line, and lower it over the side into the sea to the required depth. The pumps were then hove round until there was a free supply of air, and then stopped whilst the reading of the gauge was taken.

The re-compression chamber was tested on the Whitehead torpedo charging column, and it was found that the pressure could be brought up to 40 lbs. on the gauge in 3 minutes. There was a leak of 1 lb. per minute, or, roughly 3 cubic feet. Afterwards Drs Haldane and Rees were compressed up to about 30 lbs. in order to further test the working of the chamber.

In the afternoon both divers made a trial dive in 15 fathoms:

	Lieutenant Damant	Mr Catto
Time of descent	2 minutes	1½ minutes.
„ on bottom	1 hour	1 hour.
„ of ascent	18½ minutes	17½ minutes.
No. 5-minute stops	1 at 30 feet	1 at 20 feet.
„ 10 „ „	1 „ 10 „	1 „ 10 „

Two double pumps were used for each diver in these and the subsequent dives. The divers were perfectly comfortable in moving about on the bottom. It may be mentioned that Lieutenant Damant had not dived previously beyond about 19 fathoms, and had no experience in diving except what he had gained in his course of instruction as a gunnery officer and in experimenting at Portsmouth for the Committee. Mr Catto had much previous experience in diving work, but had never dived beyond 23 fathoms.

¹ Reprinted from the *Report of the Admiralty Committee on Deep Diving*, 1907.

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Wednesday, 22nd August,

H.M.S. *Spanker*, off Rothesay.

In the forenoon Mr Catto descended in 23 fathoms, and in the afternoon Lieutenant Damant did the same :

	Mr Catto	Lieutenant Damant
Time of descent	2 minutes	2½ minutes.
„ on the bottom	20 „	20 „
„ of ascent	35½ „	32½ „
No. 5-minute stops	4 at 50, 40, 30, 20 feet	2 at 50, 40 feet.
„ 10 „ „	1 „ 10 feet	2 „ 20, 10 „

Thursday, 23rd August,

H.M.S. *Spanker*, off Rothesay.

After testing the pumps each diver made a descent to 25 fathoms :

	Lieutenant Damant	Mr Catto
Time of descent	2 minutes	2 minutes.
„ on the bottom	18¾ „	19¼ „
„ of ascent	37¾ „	39¾ „
No. 5-minute stops	3 at 60, 45, 30 feet	3 at 50, 40, 30 feet.
„ 10 „ „	2 „ 20, 10 feet	2 „ 20, 10 feet.

Friday, 24th August,

H.M.S. *Spanker* was taken through the narrows of the Kyles of Bute and anchored off the entrance of Loch Riddon.

In the morning, after the usual tests had been applied to the pumps, Mr Catto descended in 27 fathoms, and in the afternoon Lieutenant Damant went down in a similar depth :

	Mr Catto	Lieutenant Damant
Time of descent	2 minutes	1 minute 20 seconds.
„ on the bottom	16½ „	16¾ minutes.
„ of ascent	55½ „	44¼ „
No. 5-minute stops	4 at 60, 50, 40, 30 feet	4 at 60, 50, 40, 30 feet.
„ 10 „ „	1 at 20 feet. (Diver was employed just under the ship's bottom in examining a propeller which had been slightly injured, for 19½ minutes before coming up.)	2 „ 20, 10 feet.

Saturday, 25th August,

H.M.S. *Spanker*, off Loch Riddon.

The *Spanker* shifted her position slightly, and, after the usual tests of the pumps, both divers descended in 29 fathoms of water :

	Mr Catto	Lieutenant Damant
Time of descent	3 minutes	1½ minutes.
„ on the bottom	14½ „	13½ „
„ of ascent	46 „	48½ „
No. 5-minute stops	4 at 70, 50, 40, 30 feet	4 at 66, 54, 40, 30 feet.
No. 10 „ „	2 at 20, 10 feet	2 at 20, 10 feet.

Monday 27th August,

H.M.S. *Spanker*, off Loch Riddon.

Thirty fathoms of water were obtained. Mr Catto was the diver in the morning. The pumps used were Nos. 3604 and 3593. Six men were told off for each pump, in reliefs of 5 minutes. Details of the descent :

Time	Remarks
11.22	Glass screwed up. Depth by lead line $30\frac{1}{2}$ fathoms.
11.23 $\frac{1}{4}$	Diver under water.
11.23 $\frac{1}{2}$	„ down 50 feet.
11.23 $\frac{3}{4}$	„ „ 70 „
11.24 $\frac{1}{4}$	„ „ 110 „
11.24 $\frac{1}{2}$	„ „ 150 „
11.24 $\frac{3}{4}$	„ „ 180 „ on the bottom. 1 min. 30 secs. in descending. Revolutions averaged 32 per min., but fell to 24 for a short time, owing to the great exertions that were necessary to keep the pumps going at the higher speed. Diver quite comfortable while moving about on the bottom.
11.36 $\frac{3}{4}$	Diver called up.
11.38 $\frac{1}{2}$	„ started up.
11.39 $\frac{1}{4}$	„ at 160 feet.
11.39 $\frac{3}{4}$	„ „ 140 „
11.40 $\frac{1}{4}$	„ „ 120 „
11.41	„ „ 100 „
11.41 $\frac{1}{2}$	„ „ 70 „ 1st stop. Diver employed in gymnastic exercises. One pump stopped.
11.46 $\frac{1}{2}$	Diver at 50 feet. 2nd stop.
11.51 $\frac{1}{2}$	„ „ 40 „ 3rd stop.
11.56 $\frac{1}{2}$	„ „ 30 „ 4th stop.
12.1 $\frac{1}{2}$	„ „ 20 „ 5th stop.
12.11 $\frac{1}{2}$	„ „ 10 „ 6th stop. There were no ill-effects. Water jackets gained 20 degrees F.
12.22 $\frac{1}{2}$	Diver called up.
12.23 $\frac{3}{4}$	Glass off.

Afternoon. Lieutenant Damant.

2.14 $\frac{1}{2}$	Screwed up glass.
2.15 $\frac{3}{4}$	Diver under water.
2.16	„ down 70 feet.
2.16 $\frac{1}{2}$	„ „ 120 „
2.16 $\frac{3}{4}$	„ „ 160 „
2.17	„ „ 186 „ on the bottom. 1 minute 20 seconds in descending. Revolutions averaged 30 per minute.
2.29	Diver called up.
2.30	„ started up.
2.31	„ at 170 feet.
2.33	„ „ 120 „ Diver stopped $1\frac{1}{4}$ minutes.
2.33 $\frac{1}{2}$	„ „ 70 „ 1st stop.
2.38 $\frac{1}{2}$	„ „ 50 „ 2nd „

Time	Remarks
2.43 $\frac{1}{2}$	Diver at 40 feet. 3rd stop.
2.48 $\frac{1}{2}$	„ „ 30 „ 4th „
2.53 $\frac{1}{2}$	„ „ 20 „ 5th „
3.3 $\frac{1}{2}$	„ „ 10 „ 6th „
3.13 $\frac{1}{2}$	„ called up.
3.15 $\frac{1}{4}$	Glass off. There were no ill-effects. Later in the afternoon the pumps were tested at different temperatures of the water jacket, to see how the leakage was affected.

Tuesday, 28th August.

In the same locality, Lieutenant Damant made a second descent in 30 fathoms in order to obtain samples of the air in the helmet. The pumps used were Nos. 3588 and 3592 :

Time	Remarks
10.18 $\frac{1}{2}$	Diver under water.
10.20 $\frac{1}{4}$	„ on the bottom, 1 minute 40 seconds in going down.
10.34 $\frac{1}{2}$	„ started up.
11.21 $\frac{1}{2}$	Glass off. Whilst on the bottom, diver took two samples whilst at rest. There was a distinct tide on the bottom, which affected the diver.

Analysis of Samples.

No. of sample	CO ₂ per cent.	O ₂ per cent.	CO ₂ production in cubic feet per minute
1st	·32	20·86	·025
2nd	·50	20·43	·041 (? tide)

In the afternoon Mr Catto was in the dress. Pumps Nos. 3588 and 3592 were used :

Time	Remarks
2.17	Glass screwed up.
2.18 $\frac{1}{4}$	Diver down 60 feet.
2.19	„ „ 100 „
2.19 $\frac{3}{4}$	„ „ 180 „ on the bottom. The diver took down with him a wire hawser to shackle on to a sinker.
2.31 $\frac{3}{4}$	Diver called up, but could not come up as he was foul, until—
2.48 $\frac{1}{2}$	„ started up.
2.50 $\frac{1}{2}$	„ at 140 feet.
2.53	„ „ 100 „ 1st stop.
2.56	„ „ 80 „ 2nd „
3.1	„ „ 60 „ 3rd „
3.7	„ „ 50 „ 4th „
3.12	„ „ 40 „ 5th „
3.22	„ „ 30 „ 6th „
3.37	„ „ 20 „ 7th „
3.52	„ „ 15 „ 8th „
4.0	„ „ 10 „ 9th „
4.18 $\frac{1}{2}$	„ on the surface.

Mr Catto attempted to shackle a hawser on to the sinker. He found the sinker without the slightest difficulty, and then, having tied his distance line to it, went back to the hawser. He found this in bights, and he seems to have got within the coils, and in trying to find the end of the wire to have fouled his life line. When called up he could not get away, and it was 20 minutes before he could clear himself. In all he was down $28\frac{3}{4}$ minutes in 30 fathoms of water. The rate of the pump could not be kept up above 24 revolutions per minute, and the supply of air was not adequate to his exertions to free himself, so that he was almost overcome by the excess of CO_2 . On account of his long exposure during heavy work, great care was taken in decompressing him, $1\frac{1}{2}$ hours being allowed. There were no ill-effects.

Thursday, 30th August,
H.M.S. *Spanker*, off Loch Riddon.

Mr Catto made another descent under the same conditions, and shackled on the hawser to the sinker in 4 minutes after reaching the bottom. The revolution of the pump averaged 24 to 30 per minute. The day was very bright, with the sun shining on the water, so that the diver saw with comparative ease in the water.

In the afternoon Lieutenant Damant, at the same depth, took three samples of the air in the helmet, and the pumps were tested at 180 feet pressure. He suffered from no ill-effects:

	Mr Catto	Lieutenant Damant
Time of descent	3 minutes	1 min. 20 secs.
„ on the bottom	$12\frac{3}{4}$ „	13 minutes.
„ of ascent	$46\frac{1}{4}$ „	$46\frac{1}{4}$ „
No. 5-minute stops	4 at 70, 50, 40, 30 feet	4 at 70, 50, 40, 30 feet.
„ 10 „ „	2 at 20, 10 feet	2 at 20, 10 feet.

Analysis of Samples obtained by Lieutenant Damant.

	Per cent.	First sample	Second sample	Third sample
CO_2	·43	·39	·36
O_2	20·56	20·52	20·47
Deficiency of oxygen	·48	·52	·57
CO_2 produced in cubic feet per minute		·035	·029	·027

Friday, 31st August,
H.M.S. *Spanker* moved down to the entrance of Loch Striven, where 35 fathoms of water could be obtained.

In the morning Lieutenant Damant was the diver. Pumps Nos. 2593, 3604 and 3592 were tested and used. Six hands were told off for each pump in reliefs of 5 minutes:

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Time	Remarks
11.8	Glass screwed up.
11.8 $\frac{1}{4}$	Diver under water.
11.9	„ down 80 feet.
11.9 $\frac{1}{4}$	„ „ 120 „
11.9 $\frac{1}{2}$	„ „ 150 „
11.9 $\frac{3}{4}$	„ „ 180 „
11.10 $\frac{1}{4}$	„ „ 200 „
11.10 $\frac{1}{2}$	„ „ 216 „ on the bottom. Revolutions kept at 30 per minute, and the diver had a good supply of air.
11.13 $\frac{1}{2}$	Diver took samples seated on the shot at the bottom of the rope.
11.15 $\frac{1}{2}$	„ called up.
11.16 $\frac{1}{4}$	„ started up.
11.17	„ at 190 feet.
11.18	„ „ 110 „ Diver stopped to blow off sampling tube.
11.20 $\frac{1}{2}$	„ „ 90 „ 1st stop.
11.23 $\frac{1}{2}$	„ „ 70 „ 2nd „
11.28 $\frac{1}{2}$	„ „ 52 „ 3rd „
11.33 $\frac{1}{2}$	„ „ 42 „ 4th „
11.39 $\frac{1}{2}$	„ „ 32 „ 5th „
11.44 $\frac{1}{2}$	„ „ 22 „ 6th „
11.54 $\frac{1}{2}$	„ „ 11 „ 7th „
12.4 $\frac{1}{2}$	„ called up.

There was no light on the bottom, which was of soft mud. The depth by the shot rope was 210 feet. Pressure was 93 $\frac{1}{2}$ lbs. The gauge showed a pressure of 216 feet of fresh water with the pumps stopped, and 220 feet whilst they were heaving. The actual depth, as carefully measured on the shot rope against the ship's standard measure, was just over 35 fathoms, 210 feet.

In the afternoon Mr Catto made the same descent, and reached 35 fathoms. He found that the air supply was more than ample. He walked out to the end of his distance line, and then took a sample of the air in his helmet :

Time	Remarks
2.12	Screwed up glass. Same pumps as last.
2.12 $\frac{3}{4}$	Diver under water.
2.14 $\frac{3}{4}$	„ on the bottom. Revolutions reduced to 24, as the diver found the supply too much. He proceeded to the end of his distance line before taking his sample.
2.20 $\frac{1}{2}$	Diver started up.
2.27 $\frac{3}{4}$	„ at 90 feet. 1st stop.
2.30 $\frac{1}{3}$	„ „ 70 „ 2nd „
2.35 $\frac{3}{4}$	„ „ 50 „ 3rd „
2.40 $\frac{3}{4}$	„ „ 40 „ 4th „
2.45 $\frac{3}{4}$	„ „ 30 „ 5th „
2.50 $\frac{3}{4}$	„ „ 20 „ 6th „
3.0 $\frac{3}{4}$	„ „ 10 „ 7th „
3.10 $\frac{3}{4}$	„ called up.

Analysis of Samples.

				Lieut. Damant	Mr Catto
CO ₂ per cent.	·14	·53
O ₂ „ „	20·89	20·34
Deficiency of O ₂ per cent	·15	·70

Monday, 3rd September.

Experiments on rest and measured work were carried out, by means of an arrangement of rope and pulleys by which the diver on the bottom raised and lowered a 56 lb. weight suspended in view of those on deck. The heavy rope and blocks used caused great friction and resistance.

Time	Remarks	
2.26	Diver, Mr Catto, descended.	
2.27½	„ on bottom, 142 feet.	
2.31	„ took sample sitting on the shot.	(No. 1.)
	Two pumps at 30 revolutions per minute.	
	Raised the weight 4 times 5 feet, at the rate of one lift per minute.	
2.36	„ took sample.	(No. 2.)
	Raised weight 7 times 5 feet in 5½ minutes.	
2.42	„ took sample.	(No. 3.)
2.45	„ started up.	
3.23	„ on surface, no ill-effects.	
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3.3½	„ Lieutenant Damant, started down.	
3.4½	„ down 100 feet.	
3.5	„ on bottom, 139 feet.	
4.0	„ took sample sitting on the shot.	(No. 4.)
	Two pumps at 26 revolutions.	
	Raised weight 5 times in 1¾ minutes.	
4.3	„ took sample.	(No. 5.)
	Raised weight 3 feet 18 times in 6¼ minutes.	
4.10	„ took sample. Pump 24 revolutions.	(No. 6.)
4.13½	„ started up.	
4.52½	„ at the surface. No ill-effects.	

Analysis of Samples.

CO ₂	·30 per cent.	} Mr Catto. Sample No. 1.
O ₂	20·72 „	
CO ₂	·70 „	} „ „ No. 2.
O ₂	20·29 „	
CO ₂	·71 „	} „ „ No. 3.
O ₂	20·23 „	
CO ₂	·18 „	} Lieutenant Damant. Sample No. 4.
O ₂	20·73 „	
CO ₂	·73 „	} „ „ No. 5.
O ₂	20·12 „	
CO ₂	·81 „	} „ „ No. 6.
O ₂	20·36 „	

Tuesday, 4th September.

The *Spanker* was anchored in six fathoms of water, and experiments were made on the bottom by Dr Haldane, Lieutenant Damant, and Mr Catto on the risks of blowing up. After being compressed in the air chamber to teach them to open their Eustachian tubes, Lieutenant and Commander E. V. F. R. Dugmore, Lieutenant G. N. Henson, Jack Haldane (age 13) all made descents in six fathoms of water. This was the first time that these had ever dived in a diving dress, which illustrates the usefulness of the re-compression chamber in the practical teaching of divers.

Wednesday, 5th September.

Exhaustive tests were made as to the leakage of the pumps and composition of the air, with the water jackets at various temperatures. The results are embodied in the Report. These experiments concluded the work undertaken for the Committee.

APPENDIX III.

We give here some illustrative protocols of certain important animal experiments.

1. *Comparison of stage (93 minutes) and uniform (100 minutes) decompression after 2 hours exposure at 75 lbs.*

(a) 15.3.07. Goats 3, 4, X A, XI A, XVI A, XIX A, XXV A.

Started up	10.23	
Reached 75 lbs.	11.3	
Started from 75 lbs.	12.43	
„ 31½ „	12.49	} Stage decompression. Total = 93 mins.
„ 27 „	12.59	
„ 22 „	1.14	
„ 18 „	1.29	
„ 13½ „	1.44	
„ 9 „	1.59	
„ 4½ „	2.14	
Reached 0 lbs.	2.16	

XIX A had bends left hind-leg at 2.20. X A passed urine not frothy at 2.27. Rest nil.

(b) 18.3.07. Goats XII A, XIII A, XV A, XVIII A, XX A, XXI A, XXIII A.

Started up	10.53	
Reached 75 lbs.	11.35	
Started from 75 lbs.	1.14	} Stage decompression. Total = 93 mins.
„ 31½ „	1.20	
„ 27 „	1.30	
„ 22 „	1.45	
„ 18 „	2.0	
„ 13½ „	2.15	
„ 9 „	2.30	
„ 4½ „	2.45	
Reached 0 lbs.	2.47	

XX A urine no froth at 2.54. XIII A at 2.55 seemed uneasy in hind-legs and lay down but nothing definite; at 3.0 right hind-leg slight limp and foot-drop; foot-drop very marked at 3.10 and could hardly walk; alright at 4.0. Rest nil.

(c) 14.3.07. Goats XXIV A, XXVI A, XXVII A, XXVIII A, XXIX A.

Started up	10.35	
Reached 75 lbs.	11.14	
Started from 75 lbs.	12.55	} Stage decompression. Total = 93 mins.
„ 31½ „	1.1	
„ 27 „	1.11	
„ 22½ „	1.26	
„ 18 „	1.41	
„ 13½ „	1.56	
„ 9 „	2.11	
„ 4½ „	2.26	
Reached 0 lbs.	2.28	

XXIV A showed bends left fore-leg during decompression from 4½ to 0; XXVII A passed urine no froth at 2.32; XXVIII A bends left hind-leg at 2.40. Rest nil.

(d) 12.3.07. Goats XXIV A, XXVI A, XXVII A, XXVIII A, XXIX A.

Started up	12.53	
Reached 75 lbs.	1.1	
Started from 75 lbs.	3.0	} Uniform decompression. Total = 100 mins.
Reached 60 lbs.	3.20	
„ 45 „	3.40	
„ 30 „	4.0	
„ 15 „	4.20	
„ 0 „	4.40	

XXIX A bleated at about 1 lb., gnawed side, lay down; both fore-legs completely paralysed and hind-legs unsteady; kept head bent round on left side; bleated continually; no dyspnoea till 4.55, when it was moderate; seemed like to die. At 5.10 no dyspnoea, stopped bleating, could just stand. Could walk at 5.35. XXVII A passed very frothy urine at 4.41; both hind-legs bad bends at 4.50, right fore-leg at 5.10. XXIV A left hind-leg partial foot-drop and bends at 4.50; left fore-leg bends at 5.0. XXVIII A bad bends right fore-leg, won't stand up; at 5.0 could not stand, had constant nystagmus, bleated; at 5.10 left hind-leg bad bends, nystagmus stopped, no bleating; walked very badly at 5.30. XXVI A no symptoms.

(e) 19.3.07. Goats 3, 4, X A, XI A, XVI A, XIX A, XXV A.

Started up	10.30	
Reached 75 lbs.	11.14	
Started from 75 lbs.	12.52	
Reached 60 lbs.	1.12	Uniform decompression. Total = 100 mins.
„ 45 „	1.32	
„ 30 „	1.52	
„ 15 „	2.12	
„ 0 „	2.32	

XVI A came out with bad bends left hind and right fore-legs; could hardly walk and kept head twisted round to left; much better at 2.50. XIX A urine at 2.34 full of froth; bends right fore-leg. X A began bleating at 2.38 but showed nothing till 2.44 when he had complete foot-drop right fore-leg and bends left hind-leg; at 2.50 right fore-leg paralysed, could not stand up, left fore-leg also weak; urine at 2.50 a little froth. XXV A cried out a bit, belly very tight, refuses to move, evidently far from well: died between 8 and 8.30 a.m. next day: a good many bubbles in right heart. Rest nil.

(f) 20.3.07. Goats XII A, XIII A, XV A, XVIII A, XX A, XXI A, XXIII A.

Started up	11.5	
Reached 75 lbs.	11.47	
Started from 75 lbs.	1.26	
Reached 60 lbs.	1.46	Uniform decompression. Total = 100 mins.
„ 45 „	2.6	
„ 30 „	2.26	
„ 15 „	2.46	
„ 0 „	3.6	

A goat unknown aborted two foetuses 2 in. long; they were quite warm when found, so probably during decompression. XXIII A very

frothy urine at 3.8. XIII A dyspnoea, both hind-legs dragging; at 3.25 lying down, moaning bleat, tongue and lips getting cyanosed, dyspnoea not violent. Made sure it was going to die, but at 3.55 it got up and showed only bends right fore-leg and weakness in both hind-legs. At 5.0 seemed all right. Rest nil.

(2) *The effects of a sudden drop of 51 lbs. in different parts of the scale of absolute pressure.*

(a) 26.3.07. Goats XXIV A, XXVI A, XXVII A, XXVIII A, XXIX A.

Started up	10.0
Reached 75 lbs.	10.46
Left 75 lbs.	1.23
Reached 24 lbs.	1.24½
Left 24 lbs.	2.25
,, 14 ,,	2.55
,, 8 ,,	3.18½
Reached 0 lbs.	3.21½ Total=118½ mins.

No symptoms during decompression. XXVIII A passed frothy urine at 3.23; at 3.31 had bad bends, evidently very uneasy generally; better at 4.0. XXIX A urine no froth at 3.31. Rest nil. (The immediate object of the experiment having been attained, an unwise quickening of the end of decompression gave XXVIII A bad bends.)

(b) 23.5.07. Goats 7, 9, XXX A, XXXII A, XXVII.

Start up	9.55
Reach 75 lbs.	10.35
Left 75 lbs.	1.15
Reach 24 lbs.	1.15.40''
Left 24 lbs.	2.15
,, 14 ,,	2.45
,, 8 ,,	3.5
,, 4 ,,	3.25 Total=131 mins.

No symptoms during decompression. 7 limped right hind-leg on coming out; urine 3.30 no froth. 9 right hind-leg bends at 3.35. XXXII A urine 3.35 no froth. Rest nil.

(c) 27.3.07. Goats XXIV A, XXVI A, XXVII A, XXVIII A, XXIX A.

Started up	11.0
Reached 51 lbs.	11.30
Left 51 lbs.	2.16
Reached 0 lbs.	2.20¼

XXVIII A very unsteady on hind-legs at 2.25, passed urine full of froth; legs gave way; at 2.30 lying down grunting, constant nystagmus, mucous membranes not pale; at 2.38 respiratory movements almost stopped; died 2.44 p.m. Ordinary moderate bubbling. XXVII A bends left fore-leg at 2.27, bad; a little left next day. XXIV A left hind-leg bends at 2.29; had pretty marked dyspnoea at 2.40. XXIX A urine 2.40 no froth, seemed uneasy, kept lying down but could make out nothing definite. XXVI A no symptoms.

(d) 24.5.07. Goats 7, 9, XXVII A, XXX A, XXXII A, XXVII.

Started up	9.51
Reached 51 lbs.	10.15
Started down	1.3
Reached 0 lbs.	1.7

XXX A, urine 1.10 much froth, no symptoms. XXVII A, bad bends left fore-leg, jumpy hind-legs. 7, bends right fore-leg. 9, bends right fore-leg; slight dyspnoea, bends bad, both hind-legs wobbly; dyspnoea gone by 1.40 and legs alright. XXXII A bleating, won't stand up, dyspnoea; died 1.34 p.m. Bad general bubbling. XXVII bends right fore-leg.

(e) 5.6.07. Goats XII A, XVI A, XXIII A.

Started up	9.52
Reached 45 lbs.	10.14
Left 45 lbs.	12.3
Reached 0 lbs.	12.3.33"
„ - 6 lbs.	12.10

XVI A uneasy at -5 lbs., paraplegic at 12.10, struggling and bleating, dyspnoea. XII A bends right fore-leg 12.19, bleating at 12.28. XXIII A tried to get up at 12.25 but failed once; then got up, right hind-leg paralysed; both gone just afterwards, could just crawl across tank; dyspnoea at 12.28. Raised pressure to atmospheric and opened tank at 1.10. XII A got up and seemed alright. XXIII A and XVI A lay log-like, conscious, breathing slightly and slowly. At 1.40 XXIII A could rest on fore-legs, hind-legs completely paralysed, ate hay; seemed pretty well except for paraplegia at 4.0. (Condition did not improve and it was killed six days later.) XVI A died at 3.20 p.m. A few small bubbles in right auricle and right femoral vein.

(b) 12.6.07. Goats 7, XXIV A, XXIX A, XXX A.

Started up	9.55
Reached 39 lbs.	10.13
Left 39 lbs.	12.4.20"
Reached 0 lbs.	12.4.50"
Left 0 lbs.	12.6.10"
Reached - 6 lbs.	12.11.5"

XXX A bends right fore-leg at 12.13, dyspnoea at 12.30. XXIX A bends right fore-leg at 12.14, lay down, dyspnoea at 12.24. 7 lay down, dyspnoea at 12.14. XXIV A no symptoms. Raised pressure to normal and opened up at 1.15. 7 showed bends left fore-leg and had slight dyspnoea. XXX A seemed alright. XXIV A and XXIX A were very quiet but no definite symptoms. All alright at 3.30.

APPENDIX IV.

TABLE I.

Stoppages during the ascent of a diver after ordinary limits of time from surface.

Depth		Pressure	Time from surface to beginning of ascent	Approximate time to first stop	Stoppages in minutes at different depths*						Total time for ascent in mins.
Feet	Fathoms	Pounds per square inch			60 ft.	50 ft.	40 ft.	30 ft.	20 ft.	10 ft.	
0-36	0-6	0-16	No limit ...	—	—	—	—	—	—	—	0-1
36-42	6-7	16-18½	Over 3 hours	1	—	—	—	—	—	5	6
42-48	7-8	18½-21	Up to 1 hour	—	—	—	—	—	—	—	1½
			1-3 hours ...	1½	—	—	—	—	—	5	6½
			Over 3 hours	1½	—	—	—	—	—	10	11½
48-54	8-9	21-24	Up to ½ hour	—	—	—	—	—	—	—	2
			½-1½ hours ...	2	—	—	—	—	—	5	7
			1½-3 hours ...	2	—	—	—	—	—	10	12
			Over 3 hours	2	—	—	—	—	—	20	22
			Up to 20 mins.	—	—	—	—	—	—	—	2
54-60	9-10	24-26½	20-45 mins. ...	2	—	—	—	—	—	5	7
			¾-1½ hours ...	2	—	—	—	—	—	10	12
			1½-3 hours ...	2	—	—	—	—	5	15	22
			Over 3 hours	2	—	—	—	—	10	20	32
			Up to ¼ hour	2	—	—	—	—	—	—	2
60-66	10-11	26½-29½	¼-½ hour ...	2	—	—	—	—	—	5	7
			½-1 hour	2	—	—	—	—	3	10	15
			1-2 hours ...	2	—	—	—	—	5	15	22
			2-3 hours ...	2	—	—	—	—	10	20	32
			Up to ¼ hour	2	—	—	—	—	—	2	4
66-72	11-12	29½-32	¼-½ hour ...	2	—	—	—	—	3	5	10
			½-1 hour ...	2	—	—	—	—	5	12	19
			1-2 hours ...	2	—	—	—	—	10	20	32
			Up to 20 mins.	2	—	—	—	—	—	5	7
			20-45 mins. ...	2	—	—	—	—	5	10	17
72-78	12-13	32-34½	¾-1½ hours ...	2	—	—	—	—	10	20	32
			Up to 20 mins.	2	—	—	—	—	—	5	7
			20-45 mins. ...	2	—	—	—	—	5	15	22
			¾-1½ hours ...	2	—	—	—	—	10	20	32
			Up to 20 mins.	2	—	—	—	—	—	5	7
78-84	13-14	34½-37	20-45 mins. ...	2	—	—	—	—	5	15	22
			¾-1½ hours ...	2	—	—	—	—	10	20	32
			Up to 10 mins.	2	—	—	—	—	—	3	5
			10-20 mins. ...	2	—	—	—	—	3	5	10
			20-40 mins. ...	2	—	—	—	—	5	15	22
84-90	14-15	37-40	40-60 mins. ...	2	—	—	—	3	10	15	30
			Up to 10 mins.	3	—	—	—	—	—	3	6
			10-20 mins. ...	2	—	—	—	—	3	5	10
			20-35 mins. ...	2	—	—	—	—	5	15	22
			35-55 mins. ...	2	—	—	—	3	10	15	30
90-96	15-16	40-42½	Up to 15 mins.	3	—	—	—	—	3	5	11
			15-30 mins. ...	3	—	—	—	3	7	10	23
			30-40 mins. ...	3	—	—	—	5	10	15	33
			Up to 15 mins.	3	—	—	—	2	3	7	15
			15-25 mins. ...	3	—	—	—	5	5	10	23
108-120	18-20	48-53½	25-35 mins. ...	3	—	—	—	5	10	15	33
			Up to 15 mins.	3	—	—	—	2	5	7	17
			15-30 mins. ...	3	—	—	—	5	10	15	33
			Up to 12 mins.	3	—	—	—	3	5	5	16
			12-25 mins. ...	3	—	—	2	5	10	12	32
144-156	24-26	64½-70	Up to 10 mins.	3	—	—	—	3	5	5	16
			10-20 mins. ...	3	—	—	2	5	10	12	32
			Up to 10 mins.	3	—	—	2	3	5	5	18
			10-16 mins. ...	3	—	2	3	5	7	10	30
			Up to 9 mins.	3	—	—	2	3	5	5	18
168-180	28-30	75-80½	9-14 mins. ...	3	—	2	3	5	7	10	30
180-192	30-32	80½-86	Up to 13 mins.	3	—	2	3	5	7	10	30
192-204	32-34	86-91½	Up to 12 mins.	3	2	2	3	5	7	10	32

* During each stoppage the diver should continue to move his arms and legs.

TABLE II.

Stoppages during the ascent of a diver after delay beyond the ordinary limits of time from surface.

Depth		Pressure	Time from surface to beginning of ascent	Approximate time to first stop	Stoppages in minutes at different depths								Total time for ascent in mins.	
f	Fathoms	Pounds per square inch			80 ft.	70 ft.	60 ft.	50 ft.	40 ft.	30 ft.	20 ft.	10 ft.		
66	10-11	26½-29½	Over 3 hours	2	—	—	—	—	—	—	10	30	42	
72	11-12	29½-32	{ 2-3 hours ...	2	—	—	—	—	—	—	10	30	42	
			Over 3 hours	2	—	—	—	—	—	—	20	30	52	
78	12-13	32-34½	{ 1½-2½ hours	2	—	—	—	—	—	—	20	25	47	
			Over 2½ hours	2	—	—	—	—	—	—	30	30	62	
84	13-14	34½-37	{ 1¼-2 hours ...	2	—	—	—	—	—	—	15	30	47	
			2-3 hours ...	2	—	—	—	—	—	5	30	30	67	
			Over 3 hours	2	—	—	—	—	—	10	30	35	77	
			{ 1-1½ hours ...	2	—	—	—	—	—	5	15	25	47	
90	14-15	37-40	{ 1½-2½ hours...	2	—	—	—	—	—	5	30	35	72	
			Over 2½ hours	2	—	—	—	—	—	20	35	35	92	
			{ 1-1½ hours ...	2	—	—	—	—	—	5	15	30	52	
			1½-2½ hours...	2	—	—	—	—	—	10	30	35	77	
96	15-16	40-42½	Over 2½ hours	2	—	—	—	—	—	30	35	35	102	
			{ 40-60 minutes	2	—	—	—	—	—	10	15	20	47	
108	16-18	42½-48	1-2 hours ...	2	—	—	—	—	5	15	25	35	82	
			Over 2 hours	2	—	—	—	—	15	30	35	40	122	
			35-60 minutes	2	—	—	—	—	5	10	15	25	57	
			1-2 hours ...	2	—	—	—	—	10	20	30	35	97	
120	18-20	48-53½	Over 2 hours	2	—	—	—	—	30	35	35	40	142	
			{ ½-¾ hours ...	3	—	—	—	—	5	10	15	20	53	
132	20-22	53½-59	{ ¾-1½ hours ...	3	—	—	—	—	5	10	20	30	98	
			Over 1½ hours	3	—	—	—	15	30	35	40	40	163	
			25-45 minutes	3	—	—	—	—	3	5	10	15	25	61
			{ ¾-1½ hours ...	3	—	—	—	—	10	10	20	30	35	108
144	22-24	59-64½	Over 1½ hours	3	—	—	—	—	30	30	35	40	40	178
			20-35 minutes	3	—	—	—	—	3	5	10	15	20	56
156	24-26	64½-70	35-60 minutes	3	—	—	—	—	7	10	15	30	30	95
			Over 1 hour	3	—	—	20	25	30	35	40	40	193	
			16-30 minutes	3	—	—	—	—	3	5	10	15	20	56
			{ ½-1 hour ...	3	—	—	3	10	10	15	30	30	101	
168	26-28	70-75	Over 1 hour	3	—	5	25	25	30	35	40	40	203	
			{ 14-20 minutes	3	—	—	—	3	3	7	10	15	41	
182	28-30	75-80½	20-30 minutes	3	—	—	2	2	3	10	15	25	60	
			{ ½-1 hour ...	3	—	3	3	7	10	20	30	35	111	
			Over 1 hour	3	—	15	25	30	30	35	40	40	218	
			{ 13-20 minutes	3	—	—	—	3	3	7	15	15	46	
194	30-32	80½-86	20-30 minutes	3	—	—	3	3	5	10	15	25	64	
			{ ½-1 hour ...	3	—	3	5	10	12	20	30	35	118	
			Over 1 hour	3	5	20	25	30	30	35	40	40	228	
			{ 12-20 minutes	3	—	—	3	3	5	7	10	20	51	
206	32-34	86-91½	20-30 minutes	3	—	3	3	3	5	10	20	20	67	
			{ ½-1 hour ...	3	3	3	5	10	15	20	30	35	124	
			Over 1 hour	3	15	20	25	30	30	35	40	40	238	

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PERIODICALS.

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- Collected Studies from the Research Laboratory, Department of Health, New York City.* W. H. Park, Director. Vol. I. New York: M. B. Brown, Press. 215 pages. Contains the following papers: Negri Bodies, with Special Reference to Diagnosis in Suspected Rabies, by A. W. Williams.—Recent Studies in the Diagnosis of Rabies, by D. W. Poor.—The Smear Method and Frozen Sections in the Diagnosis of Rabies, by I. van Gieson.—The Routine Methods in the Treatment and Diagnosis of Hydrophobia used in the Department of Health, by D. W. Poor.—A Preliminary Note on the Action of Radium upon Hydrophobia Virus, by C. B. Fitzpatrick and D. W. Poor.—On the Presence of Certain Bodies in the Skin and Blister Fluid from Scarlet Fever and Measles, by C. W. Field.—The Concentration of Antitoxin for Therapeutic Use, by R. B. Gibson.—Some Notes on the Concentration of Diphtheria Toxin, by E. J. Banzhaf.—Report on the Diphtheria Antitoxin Horses, by E. J. Banzhaf.—The Value of Diphtheria Antitoxin in the Treatment of Diphtheria as established by Ten Years of Trial, by W. H. Park and C. Bolduan.—Viability of Klebs-Loeffler Organisms from Dried Pseudo-membrane of a rapidly Fatal Case, by A. I. von Sholly.—Virulence of Diphtheria-like Bacilli isolated from Normal Throats of Children, by A. I. von Sholly.—A Study of Pneumococci, by W. H. Park, A. W. Williams and others.—A Study of the Pneumococcus during the Summer of 1905, by M. A. Asserson.—A Comparison of Pneumococcus Strains in Recent and Original Tests, by J. L. Berry.—The Application of the Reaction of Agglutination to the Pneumococcus, by K. R. Collins.—The Addition of Calcium Salts to Nutrient Broth. A Reliable and Convenient Method for Growing the Pneumococcus, etc., by C. Bolduan.—The Communicability of Cerebro-Spinal Meningitis and the Probable Source of Contagion, by C. Bolduan.—Epidemic Cerebro-Spinal Meningitis. (Rep. from *Ann. Rep. Board of Health*, 1871, 1872), by M. Morris.—The Frequent Occurrence of Meningococci in the Nasal Cavities of Meningitis Patients and of those in Direct Contact with them, by M. E. Goodwin and A. I. von Sholly.—The Viability of Typhoid Bacilli in Oysters, by C. W. Field.—A Comparative Study of Accurate and Roughly Estimated Dilutions of Dried Blood in the Test for Suspected Typhoid Fever, by A. I. von Sholly.—Report of Bacteriological Examination of Water Specimens for the Year 1905, by M. E. Goodwin.—Methods Employed in Disinfection, by R. J. Wilson.—Viability of Tubercle Bacilli in Dried Sputum, by A. I. von Sholly.

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EXPERIMENTS ON THE INFLUENCE OF FATNESS
ON SUSCEPTIBILITY TO CAISSON DISEASE.By A. E. BOYCOTT, M.A., D.M.
AND G. C. C. DAMANT, LIEUT. R.N.*(From the Gordon Laboratory, Guy's Hospital.)*

THE probability that fatness increases the susceptibility to compressed-air illness has been pointed out in a previous paper (this *Journal*, vol. VIII. p. 410). Vernon (1907) found that fat dissolves at body temperature rather more than five times the volume of nitrogen which is taken up by water. It follows that fat has an effective bulk some five times greater than its actual bulk. Hence the volume of blood circulating through fat or fatty organs is, relatively to the effective bulk of the tissues, much reduced. The assumption and discharge of nitrogen by the tissues while under pressure and after decompression vary with the activity of the circulation. Hence fat animals would probably take longer to become saturated with nitrogen while under pressure than similar thin animals, and would get rid of the excess gas more slowly on and after decompression. It was therefore presumed that fatness would either leave unaltered or somewhat diminish the susceptibility to caisson disease with very short exposures, and increase it with moderate and long exposures. The only experimental evidence which we were then able to bring forward was the observation that pregnant goats seemed to be particularly liable to death from compressed-air illness and appeared at the same time to be definitely fatter than non-pregnant animals on naked-eye inspection. A series of experiments were therefore instituted to examine the question directly and quantitatively. Very short exposures have not been dealt with, and the experiments have been confined to the effects of decompression

on animals nearly or completely saturated with nitrogen at the given pressure.

Considerations of convenience in the estimation of the total fat in the body made the use of small animals desirable. The objections to transferring the results of experiments on rats or guinea-pigs to human experience have been already dealt with. The general scheme of the experiments has been to sort up a series of animals by one or more decompression experiments into susceptible and non-susceptible groups, and then to estimate the total fat in both groups by Leathes' modification of Liebermann's method (Hartley, 1907). Shortly, the whole animal is dissolved in strong potash and the saponification of the fat completed with the aid of alcohol: the soap solution, or commonly a small fraction of it, is then acidified with sulphuric acid, the fatty acids and "fluff" collected in a filter, dried and extracted with petroleum ether boiling below 60° C. in a Soxhlet apparatus. After removal of the solvent, at the end under diminished pressure, the residue consists of hardly anything else but the higher fatty acids. The method has proved entirely satisfactory, skin, bones, etc. giving no trouble, and with appropriate apparatus could be applied satisfactorily to much larger animals. The great advantage is that the whole animal is reduced to a homogeneous soap solution which can be properly sampled; we much doubt whether any method of mincing would allow a fair average sample to be obtained with certainty for the actual estimation. The disadvantage of the method is that the final product contains not only the fatty acids derived from fats existing as such in the animal but also those set free from the combinations in which they do not appear as free fat either microscopically or, we may assume, as solvents of excess nitrogen. This latter portion is however quite small in proportion to the total fat. It is also probably pretty constant in absolute amount for similar animals, and, since our analyses show that the total fatty acids may amount to no more than 1 per cent. of the body weight in some rats, may for the present purpose be neglected.

The animals were not selected in any way before being subjected to experiment, and were kept on the ordinary laboratory diet. Most of them were used immediately after purchase; as will be seen the general fatness of the different series varied a good deal. The results are expressed as fatty acids per cent. of the total fresh weight of the whole animal. The figures for fat would be about 4 per cent. higher. The weights are given in grammes and the sex of each animal is also indicated. Animals marked p were pregnant.

A. *Experiments with rats.*

Series I. Rats. Pressure + 100 lbs., exposure 1 hour, decompression 5 seconds.

No symptoms			Doubtfully ill			Ill			Died		
Sex	Weight gms.	Fatty acids, %	Sex	Weight gms.	Fatty acids, %	Sex	Weight gms.	Fatty acids, %	Sex	Weight gms.	Fatty acids, %
M	135	0.8	F	95	1.0	*M	185	1.3	M	107	1.4
M	108	1.5	F	112	1.8	M	224	1.3	M	54	3.4
F	114	1.7	F	108	2.2	F	160	2.7	M	177	3.6
F	157	2.2	M	103	3.2	*M	120	2.8	M	209	3.7
M	88	2.3	M	142	5.7	F	97	3.2	M	247	4.5
F	157	2.4	F	127	5.9	*Fp	200	6.7	F	155	5.1
F	136	3.1	F	138	6.0	F	143	7.5	F	154	5.2
M	138	4.1	F	122	6.6	F	177	7.6	M	44	5.8
M	220	4.6	F	107	7.3				M	49	6.0
F	120	4.8	F	189	9.5				F	37	6.5
F	83	5.1							F	114	6.8
F	105	5.1							F	41	6.9
F	135	5.7							Fp	129	7.1
M	101	5.8							Fp	195	7.4
M	97	6.1							F	147	7.9
M	200	7.7							M	102	8.1
M	125	7.9							M	137	8.9
M	125	8.1							F	219	9.7
Av	130	4.4		124	4.9		163	4.1		129	6.0

* These animals had paraplegia: the rest of the "ill" group were clearly unwell but showed no definite local symptoms.

The general result was that the animals which did not die had about three quarters of the fat of those which died. The series was by no means homogeneous in respect of size, though the average weight of each group is much the same: if the four rats under 50 gms. are deducted the average weight of the "died" group becomes 153 gms. Dividing them up into weight groups¹ the results are as follows:

	37—49 gms.	50—99 gms.	100—149 gms.	150—199 gms.	200—247 gms.
Number of rats	4	6	27	10	7
No. which died and % fatty acid	4 : 6.3	1 : 3.4	6 : 6.7	4 : 5.3	3 : 5.5
No. which were ill and % fatty acid	0	2 : 2.1	10 : 4.9	4 : 5.3	2 : 4.0
No. which showed nothing and % fatty acid	0	3 : 4.5	11 : 4.4	2 : 2.3	2 : 6.1
Average % fatty acid	6.3	3.5	5.1	4.7	5.5

As far as these indefinite results go, they would indicate that mortality runs parallel with fatness rather than with size. The four

¹ For the correlation between weight and age in tame rats see Donaldson (1906), and on the relation between weight, age and susceptibility to caisson disease Hill and Greenwood (1908).

small rats used, all of which died, had lived with their mother since birth and were a good deal fatter than most rats of similar size which had led a more competitive existence (see Series II).

Analysed by sex we find no marked difference in susceptibility.

	No symptoms	Ill	Died	Total
Males	10	5	9	24
Females	8	13	9	30

There is however a fairly regular excess of fatness in the females.

	37-49 gms.	50-99 gms.	100-149 gms.	150-199 gms.	200-247 gms.	Total
Males	5.9 %	3.9	4.8	2.4	4.4	4.5
Females	6.7 %	3.1	5.3	5.3	8.2	5.4

The three pregnant females averaged 7.1 per cent.

Series II. Rats. Exposure 1 hour, decompression 5 seconds: in this series the animals were first tried at +80 lbs., the survivors on the following day at +90 lbs. and so on up to +120 lbs. The ultimate survivors had therefore been through five experiments.

Died at +80 lbs.:—

M 26 gms.	0.85 %	M 25.5	1.0	M 210	8.2	} Average 4.0 %
M 32.5	2.3	F 51	5.75	M 242	5.8	

Died at +90 lbs.:—

F 130	8.3
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Died at +100 lbs.:—

M 52	5.2	M 47	4.1	F 101	8.9	} Average 5.0 %
F 31	9.2	M 80	1.2			

Died at +110 lbs.:—

F 52	4.6	F 42	7.0	M 127	11.3	} Average 8.8 %
F 26	4.2	M 115	6.0	F 193	9.1	

Died at +120 lbs.:—

F 52	2.8	M 39	1.6	F 164	7.5	Average 4.0 %
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Survived:—

F 55	3.3	M 49	6.7	M 151	5.9	} Average 4.2 %
F 49	2.6	M 44	3.1	M 102	4.45	
M 38	3.2	M 65	4.1			

On analysing these results according to size and sex we find:

Weight	Number	Died	Survived	Average fatty acids per cent.		
				Died	Survived	Total
<50	12	8	4	3.8	3.9	3.8
50-99	7	5	2	3.9	3.7	3.8
100-149	5	4	1	8.6	4.4	7.8
150-199	3	2	1	8.3	5.9	7.5
>200	2	2	0	7.0	—	7.0
Total	29	21	8	5.5	4.2	5.1
Males	17	11	6	4.3	4.4	4.35
Females	12	10	2	6.7	2.95	6.1

Here again we find that mortality corresponds more with fatness than with weight or sex. The difference in the fatness of those which died and those which survived (5.5% against 4.2%) is entirely due to the great difference between dying (6.7%) and surviving (2.95%) females.

Series III. Rats. Pressure +110 lbs., exposure 1 hour, decompression 35 seconds uniformly. All the animals were killed after the experiment, and, to somewhat reduce the labour of the fat determinations, were dissolved in potash in groups.

No symptoms			Ill			Died		
	gms.	%		gms.	%		gms.	%
F	147	11.8 %	*M	110	7.2 %	M	159	14.6 %
M	136		*F	170	3.85 %	F	190	
F	106		*F	182		M	161	
F	106		F	128	1.6 %	Fp	161	
M	163	7.6 %	F	150		M	157	16.1 %
F	160					F	133	
M	165					F	98	10.8 %
F	185					M	178	
F	193	7.1 %				F	115	5.8 %
M	184					F	81	
M	182					Fp	142	13.2 %
M	72					M	125	
M	45	4.3 %				M	101	5.3 %
M	46					M	86	
F	46					F	46	3.1 %
M	55					F	55	
						M	58	
						F	41	
						F	47	
Average	124	8.3 %		148	3.5 %		112	11.7 %

In this series again the rats which died have more fat than those which lived, the whole batch being fatter than those of Series I and II. The method of pooling adopted for the estimation does not allow any further analysis, but in the quite small rats, as in Series II, fatality and fatness do not go together. Females are not more susceptible than males, 9 males dying out of 19 and 10 females out of 21.

Summary of experiments with rats.

The results of Series I, II and III may be shortly summarised as follows :

(1) The rats which died had on the whole more fat than those which survived, roughly in the proportion of 100 : 70.

(2) Middle sized and large rats tend to be fatter than small rats, and female rats than male rats: the small rats and male rats used in these experiments were not definitely less susceptible¹.

B. *Experiments with guinea-pigs.*

The results of these observations are clearly unsatisfactory. It was obvious that a better selection of the animals into susceptible and non-susceptible groups would be obtained if slower decompressions could be used. This could not be done with rats, since a decompression as short as 5 minutes never (?) produces a fatal result or definite symptoms of illness. After some preliminary experiments we used large guinea-pigs. These proved necessary, decompressions of 23 and 34 minutes by stages after 1 hour² at + 100 lbs. producing the result of killing about half the animals.

Large (400—700 grammes) guinea-pigs appear to be more susceptible to caisson disease than their size would lead one to suppose. This is correlated with their relatively low rate of respiratory exchange. We made four respiration experiments to verify the commonly accepted figure of about 1.5 grammes of CO₂ per kilo per hour.

Sex and weight in grms.	CO ₂ produced per kilo per hour
M 617, M 498, M 490, M 592, M 585	1.755 grms.
M 606, F 582	1.417 ,,
F 597, M 610	1.794 ,,
M 587, F 452	1.988 ,,
Average	1.739 ,,

On this figure, which appears to be if anything rather high, a guinea-pig of 550 grammes would only produce about 1.5 grammes of CO₂ per 1000 sq. cm. of surface per hour as against rather more than 2 grammes given off by goats, dogs, etc.

In view of the method of expressing the results as percentages of fatty acid on the crude body weight, we determined the proportion which the contents of the alimentary canal bear to the total weight in a series of 11 animals:

¹ We are sceptical about the general truth of this result.

² It is not quite certain that large guinea-pigs would be saturated in one hour. It is perhaps necessary to point out that the "stage" decompressions were not supposed to be safe: this method was used because it is less tiresome to carry out than decompression at a uniform rate.

Sex and crude weight of animal		Weight of contents of alimentary canal		
	Grammes	Grammes	=	Per cent.
M	412	55		13
F	415	53		13
F	515	57		11
M	518	54		10
F	634	81		13
F	642	75		12
M	662	100		15
F	698	109		16
F	700	101		14
F	730	111		15
M	765	81		11
Average				13

The weight of the contents of the alimentary canal has not been taken into consideration in calculating the fat content of the animals dealt with below. We found that the contents yielded no appreciable fat when treated by the method used; in most cases therefore the alimentary canal was not emptied before the animals were dissolved. In all the guinea-pig experiments, the number of determinations was reduced by pooling animal in groups of "died" and "lived." In the case of pregnant animals, the figures given include both mother and foetus. In a number of cases the foetus were actually analysed separately: their percentage fat content was always less than that of the mother except in the case of one embryo nearly at full term.

Series IV. Guinea-pigs. Pressure +100 lbs., exposure 1 hour, decompression 4 minutes uniformly.

No symptoms			Died		
	gms.	%		gms.	%
M	662	3.2	F	572	4.05
F	700		F	515	6.6
M	765	2.7	Fp	642	
M	518		F	730	5.3
			F	698	
			F	634	5.9
			M	412	
			F	415	
Average	661	3.1		577	5.8

Series V. Guinea-pigs. Pressure +100 lbs., exposure 1 hour, decompression 10 minutes uniformly.

No symptoms			Died		
	gms.	%		gms.	%
M	599	2.9	F	615	9.7
M	362	1.9	M	702	
			M	662	
			F	572	
			F	588	
Average	480	2.5		628	9.7

Series VI. Guinea-pigs. Pressure +100 lbs., exposure 1 hour, decompression in 23½ minutes in stages as follows: 100 lbs. to 40 lbs. in 1 min., then 1 min. at 40, 1 min. at 35, 1 min. at 31, 2 mins. at 26½, 3 mins. at 22½, 3 mins. at 18, 3½ mins. at 14, 4 mins. at 9, and 4 mins. at 4½. The experiment was gone through three times with each available animal, so that the ultimate survivors had lived through three decompressions. The first eight animals however in the "no symptoms" list were killed after their first decompression.

No symptoms			Ill			Died in first trial		
	gms.	%		gms	%		gms.	%
Fp	509	3·9	M	767	4·2 ¹	Fp	500	9·6
F	472		M	725	4·3 ²	Fp	742	10·5
F	509		M	672	4·7 ²	F	538	12·3
M	409	3·5	M	507	4·9 ²	M	672	7·9
M	420		M	440	4·7 ²	M	675	
M	450		M	580	3·9 ²	M	617	9·1
M	517				F	577		
M	617				M	637		
M	617	2·4				F	684	8·8
M	498					F	465	
M	490					F	584	
M	592	3·6				F	522	7·1
M	585					F	622	
M	585					Fp	455	
M	545							
F	555	5·9						
						Died in second trial		
						F	537	7·4
						M	642	
						M	762	
						Died in third trial		
						Fp	580	9·2
Average	523	3·4		615	4·5		589	8·8

¹ Paralysed.² Paralysed temporarily.

Series VII. Guinea-pigs. Pressure +100 lbs., exposure 1 hour; the animals were first decompressed three times on successive days in 50 minutes by stages, and afterwards three times in 34 minutes by stage: the ultimate residue had therefore survived six decompressions. Spacing of decompressions as follows, in minutes:

100 lbs. to 40 lbs. in	1	1
Wait at 40 lbs. for	3	2
„ 36 „	3	2
„ 31 „	4	2
„ 27 „	4	3
„ 22 „	5	3
„ 18 „	6	4
„ 14 „	8	5
„ 9 „	8	6
„ 4 „	8	6
Total	50	34

No symptoms			Ill			Died in first trial		
	gms.	%		gms.	%		gms.	%
*Fp	447	3.3	M	492	3.0 ¹	Fp	484	5.2
F	382	4.8	*Fp	405	4.8 ²	Died in fourth trial		
M	402					Fp	712	8.7
M	615	3.5				Fp	462	4.3
M	490					Died in fifth trial		
						Fp	547	8.1
Average	467	3.9		448	3.9		551	6.9

¹ Menière's disease (?) twice.
² The same once.
* Both these animals showed numerous bubbles in the amniotic fluid (none elsewhere) when they were killed 5 hours after their sixth decompression which caused no symptoms.

Summary of experiments with guinea-pigs.

- (1) The guinea-pigs which died had more fat than those which lived, roughly in the ratio of 100 : 45.
(2) There is a much greater mortality among females than males :

	No symptoms	Ill	Died	Total
Males	20	7	9	36
Females	7	1	26	34
Of which pregnant	2	0	9	11
Total	27	8	35	70

- (3) The method of pooling does not allow the exact difference in fatness between all these males and females to be ascertained, but it cannot be much different from that between survivors and dead animals. Of the 50 animals available for analysis by sex, 26 males averaged 3.7% fatty acid, 24 females 6.7%, and 9 pregnant females 7.5%.

C. Experiments with dormice.

Series VIII. Dormice. Some incomplete experiments were made with dormice in the hope of being able to compare their susceptibility just before hibernation when they were very fat and after hibernation when the fat had been used up. The experiments were made in November when the mice were beginning to be sleepy at night : owing to the high mortality, they were not carried through.

106 lbs. 1 hour, 5 seconds			
M	25 gms.	24.3 %	No symptoms
F	11	8.0	„

120 lbs. 1 hour, 5 seconds

M	21 gms.	22.8 % ₀	Died at once
M	20.5	22.3	" "
*F	10.5	2.5	Died 20 mins.
*M	9.25	3.4	" "
*M	8.0	1.9	Died 10 mins.
M	15.0	5.1	Paraplegia: dead next day.
F	13.5	10.7	No symptoms.
F	12.0	7.3	"
M	15.0	7.1	"
M	15.0	10.9	"
M	12.5	3.3	"

The animals marked * were bemused, but not fast asleep; the rest were quite lively, as were seven other small (8 to 14 gms.) dormice in the same experiment which showed no symptoms and were not killed. The two lively animals which died were much the fattest; at the same time they were the largest. The sleepy ones, all of which died, were among the smallest and least fat, and give a good illustration of the influence of a sluggish circulation. Though it is not directly germane to the subject in hand, we may mention here that we found that sleepy dormice of 10 or 12 gms. can usually be killed by 75 lbs., an exposure of 1 hour at 75 lbs. with decompression in 5 seconds, while 120 lbs. with the same exposure and decompression had hardly any effect on lively animals of the same weight. If on the other hand they were in a deep sleep, an exposure of one, and even two hours at +75 or +90 lbs. did not seem to be sufficient for them to take in enough excess gas to produce a fatal effect on quick decompression.

Summary and conclusions.

Reducing the results of Series I to VII to their simplest form, we find:

			Survived			Died		
Series	Animal	Number	Number	Average weight grammes	Average per cent. fatty acid	Number	Average weight grammes	Average per cent. fatty acid
I	Rats	54	36	136	4.5	18	129	6.0
II	„	29	8	69	4.2	21	85	5.5
III	„	40	21	130	7.0	19	112	11.7
IV	Guinea-pigs	12	4	661	3.1	8	577	5.8
V	„	7	2	480	2.5	5	628	9.7
VI	„	40	22	548	3.7	18	589	8.8
VII	„	11	7	462	3.9	4	551	6.9
Total		193	100	—	—	93	—	—

All seven series give answers in the same sense, and we may conclude that we have definite experimental evidence that *fatness*

increases the susceptibility to death from caisson disease. The regularity of the results in the guinea-pig experiments suggests that fatness is here the predominant influence in individual susceptibility. The difference in weight between the "survived" and "died" is not always in the same sense, and is in no case very large. The weight factor may therefore be relatively excluded in considering these results: indirectly it is of influence in that rats, like men, tend to become fatter as they grow older¹. Our results show pretty clearly that females are fatter than males, and, in the guinea-pig series, much more susceptible. Femality, like age, is not a quality which can *per se* have any influence on susceptibility. The increased susceptibility of females is probably simply due to their increased fatness, which tends to further increase during pregnancy. With regard to symptoms other than death our evidence is very meagre. Some symptoms, e.g. bends, may be presumed to be independent of fatness.

We do not make any suggestion that the extra gas dissolved by fat produces fatal effects by its liberation *in situ*. As far as we could make out, the immediate cause of death in these rats and guinea-pigs was the usual pulmonary air embolism. Though the fat itself usually contains many bubbles, both intravascular and among the cells, obesity doubtless favours death after long exposures because the fat acts as a reservoir of nitrogen and so keeps up the nitrogen pressure in the venous blood after decompression for a time sufficiently long for bubbles to form (see this *Journal*, vol. VIII. p. 356). If paralysis due to embolism of the spinal cord is really more frequent in fat animals—and our results are not clear on this point—we must assume a similar explanation, though it is not altogether clear that an increase in the bubbles on the venous side necessarily involves a more abundant supply of arterial emboli (*ibid.* p. 414).

The practical conclusions are clear. Really fat men should never be allowed to work in compressed air, and plump men should be excluded from high pressure caissons (e.g. over + 25 lbs.) or in diving to more than about 10 fathoms, and at this depth the time of their exposure should be curtailed. If deep diving is to be undertaken, or caissons worked at pressures approximating to + 45 lbs., skinny men should be selected. It is unfortunate that an increase of experience and skill in technical operations should so often be associated with the increase in waist measurement which accompanies the onset of middle

¹ Probably also guinea-pigs. We only have figures for six small guinea-pigs (154–208 grammes) which averaged 2.9 % for males and 2.8 % for females.

life. Middle aged men have a lower rate of respiratory exchange than young men: if fatness is not the explanation of this, they are at a double disadvantage, and the two factors must be multiplied, rather than added, together.

We would take this opportunity of thanking the Governing Body of the Lister Institute and Messrs Siebe, Gorman & Co. for the loan of apparatus. The expenses were in part defrayed by a grant from the Government Grant Committee of the Royal Society.

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ABNORMAL REACTIONS TO HORSE SERUM IN THE SERUM TREATMENT OF CEREBROSPINAL FEVER.

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IN the *Journal of Hygiene* for January, 1907, I noted the effect of repeated injections of horse serum in a number of persons who were for the most part diphtheria patients. Certain of these persons developed abnormal serum reactions, and the view was expressed that the interval of time between the injections of serum was a primary factor in determining these abnormal reactions.

Introductory.

It will be recalled that the normal serum reaction, the most obvious detail of which is a skin eruption, follows injection of serum after a lapse of time, varying on either side of ten days, which is known as the period of incubation or latent period. The abnormal serum reaction as here understood differs from the normal in its more speedy onset and more rapid course. It is of two forms, the immediate and the accelerated. The immediate form may be local or general, appearing in 24 hours or less. The accelerated form succeeds the injection which causes it after a shorter latent period than in the normal reaction.

Within recent months, the subject in various aspects has been further under consideration, with reference to animals by Rosenau and Anderson (VII. and XI. 1907), Otto (1907), Goodman (VI. 1907) and others, and with reference to man by Goodall (VII. 1907). The conclusions of my paper are in general sustained by Goodall's observations, which likewise relate to diphtheria patients. His earliest immediate reaction after reinjection for diphtheria was 35 days from the initial puncture, a period longer by 24 days than was shown by my plague contact. His

earliest accelerated reaction after reinjection for diphtheria was 25 days after the first injection, a period shorter by 16 days than in my case VII. 86. Goodall further suggests that, in diphtheria, original normal reactions and original large doses predispose to subsequent abnormal reactions.

It is here proposed to note the corresponding serum phenomena which emerged during the treatment of certain cases of cerebrospinal fever. In view of the suggestion referred to later that diphtheria poison plays a contributory part in producing supersensitisation of guinea-pigs, it may be profitable to review a series of cases exempt from the influence of that toxin. Further, since the frequency of dosage, and the volume of serum given, in a number of these subjects of cerebrospinal fever, were higher than is customary in dealing with diphtheria, it may be of interest to observe whether there were any corresponding differences in the character of the reactions induced.

Sera employed.

The four curative sera employed were derived from the horse. The serum first used, Prof. A. Wassermann's, was obtained from the Royal Prussian Institute for Infectious Diseases. The serum which was chiefly employed was prepared by Messrs Burroughs, Wellcome & Co. Messrs Rebman supplied the third of the sera, and the fourth was furnished by Messrs Meister Lucius and Brüning of Höchst am Maine. Large doses and frequent administration were associated with Messrs Burroughs, Wellcome & Co's serum especially, and with Messrs Rebman's serum in less degree. The serum from Höchst was little used for this series of cases, and the doses of Prof. Wassermann's serum which were given were relatively small. So far as serum reactions are concerned the four sera did not show specific differences, and it will not be necessary to distinguish between them in reporting the results of their repeated administration.

Survey of the cases.

Of the cases of cerebrospinal fever admitted to Belvidere Hospital during parts of 1906 and 1907, 73 were injected with serum on more than one occasion. Of the 73 cases 23 died within 10 days of the first injection, without showing any serum reaction, either normal or abnormal. The 50 remaining cases form the material for these remarks.

The 50 cases had 270 injections in all, an average of 5·4 per head. Twenty-eight of the 50 had more than three injections, 17 had more than five, and six had more than 10. The largest number of injections in a single case was 21. In my earlier paper (1. 1907), among 135 cases of repeated injection for diphtheria, only two had more than three administrations.

The 50 cases received 7958 c.c. of serum in all, an average of 159 c.c. per head. Twenty-three of the 50 had more than 90 c.c. and 12 had more than 200. Six of the cases had each more than half a litre in all, the largest total for a single person being 725 c.c. In my earlier paper (1. 1907), among 135 cases of diphtheria, 33 had more than 90 c.c. These figures are the basis for the statement made above that both as regards frequency and volume of dosage the standard of the 50 cases of cerebrospinal fever was higher than is usual in diphtheria.

Of the 50 cases 36 had subcutaneous injection only; in 13 cases one injection was into the spinal theca, and the remaining injections were subcutaneous; and in one case, one injection was thecal, one intravenous and the remainder subcutaneous. None of the cases furnished a record of previous serum treatment at any time. Twenty-six recovered from the disease.

Of the 50 cases 29 had serum rashes. In 15 of these the normal rash alone appeared. Seven of the patients showed both a normal and an abnormal reaction, and seven had an abnormal reaction only. A normal reaction with or without a following abnormal reaction was thus observed in 22 cases, and an abnormal reaction, with or without a preceding normal reaction, in 14 cases.

Points for consideration.

In dealing with the influences at work on these cases in producing abnormal reactions, the five following points may be considered:—the total quantity of serum injected; the total number of injections given; the interval between the first injection and the final injection; the presence or absence of an earlier normal reaction; and the quantity of serum administered within the latent period of the normal reaction.

Quantity of serum.

The dosage in 27 of the 50 cases was 100 c.c. or less, while 23 cases had more than 100 c.c. Of the 27 cases which had 100 c.c. or less, three—or 11·1 per cent.—showed an abnormal reaction, and of the 23

cases which had more than 100 c.c., 11—or 47·8 per cent.—had an abnormal reaction. Of the 27 cases however which had less than 100 c.c. 19 received the last injection within the latent period, and eight beyond it. The three cases of abnormal reaction were all among the eight who received their last injection after the close of the latent period, a fact which suggests that the time between injections was at least as potent in causing abnormal reactions as the volume of serum administered. These relations are maintained in Fig. 1 which shows in diagrammatic form the relative incidence of normal and abnormal reactions in the 50 cases as the quantity of serum administered rises from 50 c.c. to 100 c.c. and thence by gradations of 100 to over 700 c.c. The normal reaction is seen to increase slightly in frequency as the dosage rises: the incidence of the abnormal reaction is irregular and its infrequency in the lower groups, as just stated, is associated with a factor which is related to time rather than volume. Fig. 1 thus fails to show that increase of the total quantity of serum given induces a corresponding increase in the frequency of abnormal reactions.

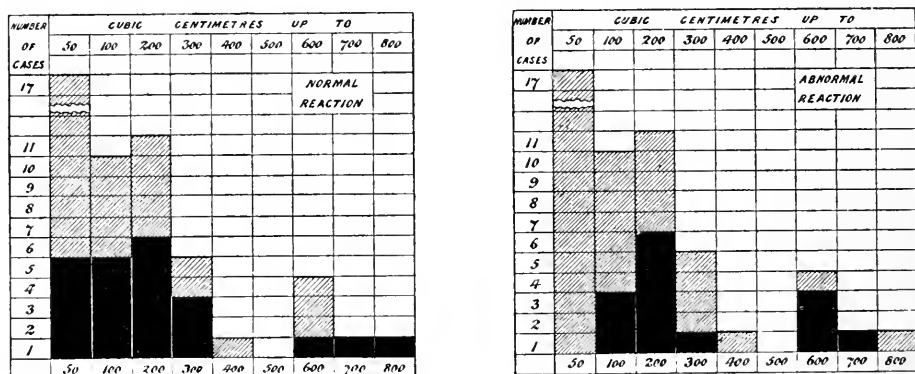


Fig. 1.

Number of injections.

The number of injections given to 33 of the 50 cases was five or less, while 17 had more than five injections. Of the 33 cases which had five injections or less, three—or 9 per cent.—exhibited an abnormal reaction, and of the 17 cases which had more than five injections 11—or 64·7 per cent.—were cases with an abnormal reaction. Frequency of administration however cannot with certainty be regarded as determining an abnormal reaction if the interval of time between the effective injection and the injection which immediately precedes is partly within and partly without the latent period of the normal serum

reaction, counted from the first injection of all. If, for example, in any particular case a series of injections be given within 10 days of the first injection, and if the series of injections be followed at a later date by another injection which induces an abnormal rash, it may well be that this result is not due to frequency of injection but to the circumstance that the final injection took place beyond the latent period of the normal reaction. This statement affects alike cases with five injections or less and cases with more than five injections. Of the 33 cases which had five injections or less, three—Nos. 1, 26, and 34—had abnormal reactions. In all three the interval between the effective injection and the injection immediately preceding it was partly within and partly without the latent period of the normal reaction. Of the 17 cases which had more than five injections 11 had abnormal reactions. Three cases of the 11—Nos. 12, 32 and 36—had an interval similar to the above. In the remaining eight—Nos. 7, 10, 11, 17, 21, 28, 31 and 40—the interval between the effective injection and the injection immediately preceding was entirely without the average latent period. In three of these cases—Nos. 21, 31 and 40—the interval was demonstrably without the actual latent period, as the close of the latent period in each case had been marked by a normal reaction. Case 21 which had six injections within the latent period, had the first abnormal reaction after the tenth injection; case 31 which had three injections within the latent period had the abnormal reaction after the seventh injection; and case 40 which had six injections within the latent period had the first abnormal reaction after the ninth injection. In certain cases therefore by continuing to inject, an abnormal reaction may at length be obtained, after preceding administrations outwith the latent period have failed to induce it. That is to say that in certain cases the number of injections is a factor in determining abnormal reactions.

The number of injections in the 50 cases is arranged in diagrammatic form in Fig. 2, which shows the relative incidence of normal and abnormal reactions respectively as the number of injections increases from two to over 20. There is no evidence from the diagram that the frequency of the normal reaction increases with the number of injections of serum. The preponderance of abnormal reactions in the higher groups is to be looked on as subject to the reservation above noted regarding the coincidental influence of the latent period. The most that can be said is already stated, so far as these cases are concerned; under certain conditions the number of injections is a factor in inducing abnormal reactions.

Interval of time.

When the 50 cases are considered with special reference to the interval of time between the first injection of all and the injection which induced an abnormal reaction if an abnormal reaction was induced, or the last injection given if no abnormal reaction occurred, the importance of this interval, already referred to incidentally, comes clearly under notice. In 24 of the 50 cases there was a period of 10 days or less between the first injection and the injection which induced an abnormal reaction, or the last injection given if no abnormal reaction occurred. In 26 of the cases the corresponding period was over 10 days. Of the 24 cases within the 10 day interval none had abnormal reactions. Of the 26 cases beyond the 10 day interval, 14—or 53·8 per cent.—had abnormal reactions.

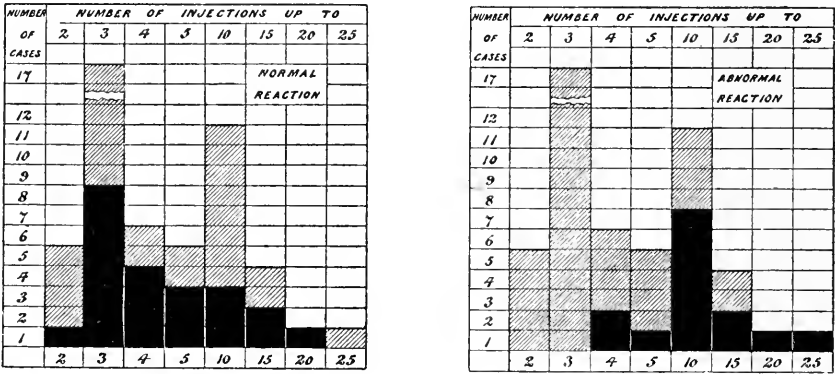


Fig. 2.

The intervals between the first injection of all and the injection which led to an abnormal reaction, or the last injection given if no abnormal reaction occurred, are arranged in diagrammatic form in Fig. 3, which shows the relative incidence of normal and abnormal reactions as the intervals lengthen from two days to over 60. The normal reaction is seen to be constant throughout the groups. It has no apparent relation to the length of the interval under consideration; in many cases it occurred in the course of it. The abnormal reaction on the other hand is absent from the columns showing intervals up to two and ten days respectively. It first appears in the column with intervals between 11 and 20 days, but it will be noted that it shows no definite tendency to increase in frequency as the intervals lengthen further. These observations accord with the results of my previous paper. They suggest that the primary condition for an abnormal reaction is the

lapse of a certain interval of time, but that further lapse of time beyond this interval has no additional influence in inducing the phenomenon. The interval in question which is approximately ten days is the latent period of the normal reaction.

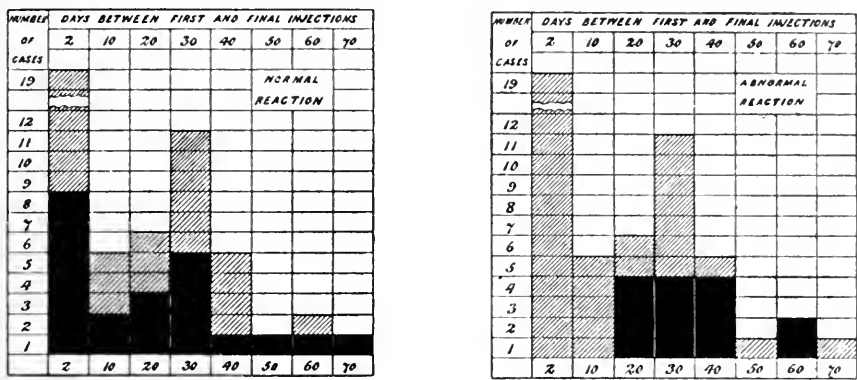


Fig. 3.

Preceding normal reaction.

As already noted Goodall (VII. 1907) has suggested that a patient who has had a serum reaction at a primary attack of diphtheria is more likely than a patient who has not had such a reaction to develop an abnormal reaction after re-injection for a second attack. Corresponding relations for the 50 cases of cerebrospinal fever are shown in the following:

Table 1.

Normal reaction	Abnormal reaction		Total
	Present	Absent	
Present	7	15	22
Absent	7	21	28
			50

Of 22 cases with a normal reaction at the first, seven—or 31·8 per cent.—furnished an abnormal reaction at a later period. Of 28 cases which did not exhibit a normal reaction at the first, seven—or 25 per cent.—had a normal reaction subsequently. The difference between these percentages is in the sense of Goodall's conclusion: it is however too slight to have significance.

Preceding large doses of serum.

Goodall has also indicated that the greater the quantity of serum administered during the primary attack of diphtheria, the more likely is an abnormal reaction to occur after serum for a second attack. While Goodall's patients received the injection of serum which led to an abnormal reaction on account of a relapse or second seizure of diphtheria, the corresponding doses for the 50 cases now under notice were given in the routine treatment of a single attack of cerebrospinal fever, or on account of oscillations in its course, and therefore do not lend themselves to an equally ready classification. The close of the latent period of the normal reaction has thus been selected as a line of division, and the presence or absence of abnormal appearances has been considered with reference to the number of injections which occurred within the period in question. Table 2 shows the incidence of abnormal reactions as the number of injections within the latent period rises from one to seven. The cases however are unequally divided among the groups and no deduction seems justified.

Table 2.

Number of injections of serum within the latent period	Abnormal reaction		Total
	Present	Absent	
1	2	—	2
2	1	6	7
3	8	26	34
4	—	1	1
5	1	2	3
6	2	—	2
7	—	1	1
Total	14	36	50

That the 50 cases do not definitely support Goodall's view that original normal reactions and original large doses predispose to subsequent abnormal reactions is possibly a consequence of the smallness of the numbers involved: it will however be recalled that Otto and Rosenau and Anderson (IV. 1906) have published experiments to the effect that guinea-pigs which have been used for standardising purposes, and which have for that reason been injected with horse serum and diphtheria toxin together, are more prone to furnish abnormal reactions in response to following injections of serum than guinea-pigs which have been treated for experimental objects with normal horse serum alone. Such experiments suggest to Otto, and also to Lewis (1908),—though Rosenau

and Anderson in a later paper (VII. 1907) do not entirely concur—that diphtheria toxin plays a part in sensitizing animals to the serum of animals of another species; and it is matter for speculation whether diphtheria patients, containing as they do in their bodies the poison of that disease, may not be more favourable subjects for abnormal serum phenomena than persons who are suffering from cerebrospinal fever. Several observers have already commented on the neutral qualities of the anti-meningococcic sera, so far as specific serum effects are concerned. Wassermann (1907), for example, dealing with 102 cases which were treated with serum, for the most part in repeated doses, reports appearances of the nature of nettlerash in five examples only.

The prevailing severity of cerebrospinal fever may also be adduced as a cause for the slight proportion of abnormal reactions in cases which had showed normal reactions at the first. Although there was no special mortality among cases which had a normal reaction at the first and no abnormal reaction later, yet many who ultimately recovered were in the extreme of illness during the period when abnormal reactions were possible, and their exhausted condition at that time may well have had an influence in suppressing the reactions in question.

Details.

Since any value which may attach to these remarks must depend on the interpretation which has been placed on the reactions shown by the cases, details are added relating to the reactions in question. Most of the facts are suitable for arrangement in tabular form and are so arranged in Table 3. The abnormal reactions of individual cases are separately described. In the table and in the text S_1 means the first injection of serum, S_2 the second injection, and so on for higher numbers.

Details of abnormal reactions.

Case 1. Accelerated reaction following S_3 , S_4 or S_5 on 6th, 5th, or 4th day. A slight urticaria of one day's duration as opposed to the two days' duration of the preceding normal rash.

Case 7. Immediate reaction, thrice. *First* after S_{10} on 52nd day, an area of erythema appearing 30 minutes after injection, measuring 12 cm. in diameter, and lasting 2 hours. *Second.* After S_{11} on 53rd day, an area of erythema appearing 15 minutes after injection, measuring 12 cm. in diameter and lasting $2\frac{1}{2}$ hours. *Third.* After S_{12} , on 54th day, an area of erythema appearing 15 minutes after injection, measuring 2 cm. in diameter, and lasting $1\frac{1}{2}$ hours. A gradual diminution in the activity of the reaction, as if S_{10} and S_{11} had each neutralized a certain

24	M	16	300	6	1	2	3	8	10	11	7	Erythema and urticaria, lasting 4 days	0
25	M	3	150	5	1	2	4	30	67		3	Fugitive erythema, lasting 2 days, followed on 9th day by urticaria of 3 days' duration, and on 28th day by slight erythema	0
26	M	5	125	4	1	2	3	26			2	Fugitive erythema, lasting 2 days	Immediate
27	F	$1\frac{1}{2}$	75	3	1	2	3	26	27	28	7	Local erythema, lasting 6 hours	0
28	M	$1\frac{1}{2}$	250	6	1	2	3	25					Immediate
29	M	9	80	4	1	2	3						0
30	M	13	225	3	1	2	3	19	23	27	9	General urticaria, lasting a few hours	0
31	F	2	175	7	1	2	3	20	22	25	9	Urticaria of 2 days' duration, followed on 16th day by urticaria of one day's duration	Immediate
32	F	3	135	6	1	2	3	18	23		8	General urticaria, lasting 3 days, followed on 14th day by urticaria lasting 12 hours	21, 23
33	F	$1\frac{1}{2}$	110	5	1	2	3	20	22	23	7	General urticaria, lasting 3 days, followed on 14th day by general urticaria, lasting 1 day	0
34	F	2	85	4	1	2	4	14	15	33	8	Local urticaria lasting 3 days, followed on 14th day by general urticaria, lasting 1 day	Immediate
35	F	$2\frac{1}{2}$	175	5	1	2	4	31	32		11	General urticaria, lasting 5 days. Temperature 102°	0
36	F	$1\frac{1}{2}$	150	6	1	2	3	4	8	9			Accelerated
37	M	19	225	3	1	2	3	9	18				0
38	M	10	600	12	1	2	3	4	8	9	11	Local urticaria, lasting a few hours	0
39	M	25	725	12	1	2	3	5	7	8	9	General urticaria, lasting 6 days	0
40	M	$2\frac{1}{2}$	645	16	1	2	3	3	5				18, 21
41	M	20	250	5	1	1	2	3	5				Immediate
42	M	26	325	3	1	2	3						0
43	F	31	60	3	1	2	3						0
44	M	3	30	3	1	2	3				10	Erythematous and morbilliform, lasting 10 days. Submaxillary, parotid and cervical adenitis. Arthritic pains of shoulders and elbows	0
45	F	$1\frac{1}{2}$	30	3	1	2	3				10	Urticaria of 24 hours' duration	0
46	F	6	20	2	1	3							0
47	F	16	60	3	1	2	3						0
48	F	$3\frac{1}{2}$	30	3	1	2	3						0
49	M	4	30	3	1	3	4						0
50	F	5	40	4	1	3	4	25			8	Erythema of 2 days' duration	0

proportion of a specific antibody present when S_{10} was given. A fall in the precipitating power of the blood of a somewhat similar character is referred to by Nicolle and Abt (II. 1908). Compare case 21. Preceding injections negative.

Case 10. Immediate reaction, thrice. First after S_4 on 33rd day, an area of erythema appearing 30 minutes after injection, measuring 20 cm. in diameter and lasting 12 hours. *Second.* After S_5 on 34th day, the same as the above. *Third.* After S_6 on 35th day, also the same as the above. Preceding injections negative.

Case 11. Immediate reaction, thrice. First after S_6 on 35th day, an urticaria appearing within 24 hours of injection and lasting 6 hours. *Second,* after S_8 on 41st day, an area of erythema appearing 15 minutes after injection, measuring 10 cm. in diameter and lasting one hour. *Third,* after S_9 on 42nd day an area of erythema, appearing 10 minutes after injection, measuring 10 cm. in diameter and lasting $1\frac{1}{2}$ hours. Preceding injections negative.

Case 12. Immediate reaction. After S_7 on 18th day, a general urticaria, appearing several hours after S_7 and lasting 4 hours.

Case 17. Accelerated reaction. Severe articular pain in shoulders on 33rd day, two days after S_{15} , and again articular pain in legs on 44th day, two days after S_{21} . The shoulder arthritis suggests an accelerated reaction: the character of the leg arthritis is less definite.

Case 21. Immediate reaction four times after a normal reaction on 15th day. *First* after S_{10} on 32nd day, an erythema appearing immediately after injection measuring 14 cm. in diameter and lasting four hours. *Second,* after S_{11} on 33rd day an erythema appearing immediately after injection measuring 12 cm. in diameter and lasting $4\frac{1}{2}$ hours. *Third,* after S_{12} on 34th day, an erythema appearing immediately after injection, measuring 4 cm. in diameter and lasting 5 hours. *Fourth,* after S_{13} on 44th day, an erythema, appearing 30 minutes after injection, measuring 2 cm. in diameter and lasting $4\frac{1}{2}$ hours. Gradual diminution in activity of reaction. Compare case 7. Preceding injections negative.

Case 26. Immediate reaction, succeeding a reaction on 2nd day which has been taken as normal. After S_4 on 26th day, redness, swelling and tenderness of the whole abdomen, appearing from 2 to 3 hours after S_4 and lasting about 12 hours.

Case 28. Immediate reaction, on 28th day, after S_5 but before S_6 , a general urticaria, appearing within 24 hours of injection, and lasting 24 hours.

Case 31. Immediate reaction. A normal reaction occurred on the 9th and 16th days as stated in Table. S_4 was on the 19th day and was followed on the 20th day by a general morbilliform rash of 24 hours' duration. S_5 was on the 23rd day and was attended by a general morbilliform rash of three days' duration. These morbilliform eruptions may have been continuations of the normal reaction, but S_7 , which was given on the 59th day, was followed within 24 hours by an erythema which affected the whole trunk: at the same time the abdominal wall became hard and swollen. This manifestation which lasted 1 day had the character of an immediate reaction.

Case 32. Immediate reaction twice, succeeding a normal reaction on 8th day. *First,* on 21st day following S_4 an abdominal urticaria, appearing within 24 hours of

injection and lasting 12 hours. *Second*, after S_5 on the 23rd day an urticaria of abdomen and legs, appearing within 24 hours of injection, and lasting 12 hours. Accepted as immediate reactions.

Case 34. Immediate reaction. Succeeding a normal reaction on 8th day. A general urticaria appearing on 21st day, within 24 hours of S_4 and lasting one day.

Case 36. Accelerated reaction. On 36th day, three days after S_6 an abdominal urticaria.

Case 40. Immediate reaction, twice, succeeding normal reaction on 9th day. *First*, after S_9 on 18th day, general erythema appearing about 12 hours after injection and lasting six hours. *Second* after S_{11} on 21st day, general erythema appearing within 24 hours of injection, and lasting one day; reappearing again on the 24th day, and lasting again for one day. Accepted as immediate reaction.

Theoretical Note.

In my earlier paper (I. 1907) I had suggested that a secondary antibody might play a part in the reactions which follow the injection of extraneous sera, inasmuch as the toxic product, which resulted from the interaction of a substance contained in the serum injected and of an antibody which it originated, might evoke a secondary antibody which combined with the toxic product, controlled its effects and ultimately brought the reaction to a close. The latent period of the secondary antibody was to be regarded as shorter than that of the primary antibody. In the normal reaction the various processes were gradual. In one of the forms of the abnormal reaction the case was otherwise; in the immediate abnormal reaction the primary antibody produced by the first injection of serum persisted at the time of the second injection, but the secondary antibody, evoked by the toxic product of the first injection of serum with the primary antibody, had already vanished from the system. When therefore the antibody-producing substance of the second injection of serum reacted with the primary antibody produced in the organism by the first injection of serum, the abruptly liberated toxic material exerted its hurtful influence unchecked until sufficient time had elapsed to admit of the preparation anew of a secondary antibody to control its effects.

Discussing this suggestion in connection with supersensitisation Goodman (VI. 1907) expresses the view that, although it may account for the phenomena occasioned by repeated injections of serum, it cannot hold for the corresponding phenomena elicited by *diphtheria toxin*. That the suggestion, which deals with a substance that is bland at the time of injection,

is not appropriate to the effects of the diphtheria poison which is toxic at the time of injection may be readily admitted, but it must at the same time be maintained that Goodman's alternative theory, if relevant as regards supersensitisation by diphtheria toxin, cannot be looked on as applicable to serum rashes. In terms of the side-chain hypothesis Goodman states that the injection of small doses of toxin leads to the destruction of certain sessile receptors and the sympathetic degeneration of others, so that vital cells are left more open to attack. Be this as it may for diphtheria toxin, there seems no occasion to suppose that reactions of supersensitisation to extraneous sera have any special relation to sessile receptors of the cells of vital organs. In my earlier paper I spoke of sessile receptors in this connection. The data then seemed somewhat equivocal. Recently however more definite evidence has become available. Rosenau and Anderson (VII. 1907) have confirmed a statement previously made by them (IV. 1906) as to the congenital supersensitiveness of the young of supersensitized female guinea-pigs,—an indication that the active substance, to some extent at least, is free in the maternal blood. Vaughan and Wheeler (VI. 1907) have referred to a similar condition in the young of animals supersensitized to egg albumen. Otto (1907) has shown that extracts of the organs of supersensitized guinea-pigs do not modify the action of horse serum on other supersensitized animals, and both Otto and Friedemann (1907) have been able to induce passive supersensitisation by injecting the serum of supersensitized animals. The general sense of these observations is that the active substance is not attached to cells in vital organs but is free in the blood of animals. There is therefore reason to think that the reactions under discussion are related less to sessile receptors of vital cells than to free receptors. The experiments cited are concerned with immediate reactions of animals; it is probable by analogy that similar conditions obtain in the human subject.

Interest also attaches to the question of a secondary antibody in the light of papers by Nicolle and Pozerski (I. 1908) and Nicolle and Abt (II. 1908). Nicolle and Abt hold that *two antibodies* play a part in serum reactions, but both antibodies in their view are primary. Though each antibody is active at a different time from the other, both are called into existence by the original dose of the extraneous material. One of the antibodies is an albuminocoagulin—or precipitin;—the other is an albuminolysin, a conception on the analogy of cytolysin. With reference to albuminocoagulin, though precipitation is not effected

within the living body, it is held that there takes place in the body a coagulation or condensation of the foreign albumen, a process by which its potential activity is lessened. Albuminolysin, again, becomes active after albuminocoagulin: it dissolves the compound of extraneous serum and albuminocoagulin and liberates a poison of the nature of endotoxin, a class of substance, as Wolff (1904) pointed out, against which the animal body has no defence. Hence the action of albuminolysin is injurious.

According to Nicolle and Pozerski (I. 1908) large doses of extraneous serum evoke albuminocoagulin; while small doses favour the production of albuminolysin. Large doses therefore should tend to mild serum reactions, and small doses to severe reactions. In animal experiments this holds good: Otto and Rosenau and Anderson (VII. 1906) have found that initial small doses are more dangerous to guinea-pigs than initial large doses. In the human subject however the reverse appears to obtain: von Pirquet and Schick (1905) and Goodall (VII. 1907) assert that in man large doses of serum are more active than small in predisposing to abnormal reactions.

With further reference to the theory that the predominance of one or other of two primary antibodies determines the nature of abnormal reactions; since albuminocoagulin—or precipitin—in excess leads to mildness or abeyance of abnormal reactions while albuminolysin in excess predisposes to their severity, it might have been expected that animals or persons whose blood was found to contain precipitin would have their serum reaction mild or absent, while those whose blood was free from precipitin would show an active response. Such an association however has not been proved to exist. On the contrary, from experiments reported by von Pirquet and Schick (1905), it would appear that there is no definite relationship between precipitin formation and the serum reaction: they do not accompany one another of necessity, nor does the presence of one imply the absence of the other. There are therefore points in the theory that two primary antibodies determine serum reactions, which are difficult to reconcile with the facts under notice.

The work of Vaughan and Wheeler (VI. 1907) with egg-albumen has a reference to the possibility of a *secondary antibody* under conditions analogous to those of the serum reaction. These observers have split egg-albumen in vitro into two portions, of which one was bland when injected into animals, and the other toxic. Both the bland portion, and entire egg-albumen itself, proved capable of sensitizing

animals to entire egg-albumen; and these phenomena Vaughan and Wheeler interpret in the sense that a material evoked by the non-toxic portion splits entire egg-albumen within the body and liberates a toxic substance which is allied or identical with the toxic extract produced in vitro. Repeated injections of the toxic extract induced some increase of the resistance of animals to it. Though the attempt to detect an antibody has failed, as Vaughan and Wheeler state, yet acquired resistance to what is probably a proteid substance suggests to the mind antibody formation. The toxic substance derived by the cleavage of egg-albumen in vivo, being similar to that obtained in vitro, may be presumed to react in a similar manner and to evoke a similar antibody.

On this analogy the toxic substance which manifests its presence by serum rashes and the like, and which is to be regarded as liberated by the action on extraneous serum of an antibody to that serum, may be expected to lead to the formation of another antibody, secondary in the sense that it is not elaborated in direct response to an antibody-producing material in the serum injected but in response to a toxic product of the reaction of that original antibody-producing material with an antibody which it gives rise to after injection.

Summary.

The following general statements are applicable to the 50 cases of cerebrospinal fever which have been under notice here.

The total volume of serum given did not affect the frequency of abnormal reactions.

The total number of injections of serum, in certain cases, may have been a factor in inducing abnormal reactions.

The interval of time between the injections concerned was the primary influence in determining abnormal reactions.

It was not apparent that a preceding normal reaction predisposed to a subsequent abnormal reaction.

It was not apparent that a large administration of serum within the latent period predisposed to a subsequent abnormal reaction.

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THE CAUSE OF LEAD POISONING IN THE TINNING OF METALS.

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IN 1901 Miss Anderson (H.M. Principal Lady Inspector of Factories) and Dr Legge (H.M. Medical Inspector of Factories) were instructed by H.M. Chief Inspector of Factories to investigate the conditions of labour in the Tinning of metals and metal articles with a view to the preparation of regulations for the conduct of this industry. Difficulties having arisen in assigning a cause for the plumbism undoubtedly existing among workers, and preparation of regulations appearing impossible without some knowledge of the source of illness, the writer was instructed to assist them experimentally in determining the causes.

The results of the enquiry are contained in a report recently issued¹.

¹ *Tinning of Metals. Special report on dangerous or injurious processes in the coating of metal with lead or a mixture of lead and tin.* By Miss A. M. Anderson (H.M. Principal Lady Inspector of Factories) and T. M. Legge, M.D. (H.M. Medical Inspector of Factories). Together with a report on an experimental investigation into the conditions of work in tinning workshops by G. Elmhirst Duckering (one of H.M. Inspectors of Factories). Cd 3793. Price 1/-. Wyman and Sons, Ltd., Fetter Lane, E.C.

The present paper contains an account of the most important of the experiments carried out during the investigation and of their results.

Process and Trades affected.

The process of Tinning may be defined as "The coating of metal articles with lead or tin or a mixture of these metals." The objects aimed at are—(a) Protection of the metal from atmospheric influences, *i.e.* oxidation, corrosion, etc. (b) The rendering of vessels built up from component parts airtight and watertight. (c) Making subsequent operations of soldering more easy. (d) The joining together of loose parts. (e) The giving of a finished appearance to articles. Tinning for purposes described in *a*, *b*, *c* and *d* is of very real utility and there is strong evidence of extension of the process to various trades. It may be pointed out also that articles of iron or steel not previously tinned must be pickled, *i.e.* treated with acids, before soldering can be successful, whereas there is no necessity for pickling if they have been already tinned. This is of great importance where articles are produced by soldering together pieces which are made by separate operations.

In this paper only tinning with a mixture of lead and tin is dealt with and this has a wide application in the manufacture of—(1) Holloware including cooking utensils, kettles, iron drums, etc. (2) Harness furniture including the plating of harness, bits, buckles, etc. (3) Brass cocks and parts of gas and water fittings. (4) Cycles and motor cars, *i.e.* tinning of rims, radiators, etc. (5) Tin plates, *i.e.* the making of terne plates. (6) Boiler tubes.

The process of tinning is the same in principle in all cases though the details vary. The article, if made of iron or steel, is "pickled"—*i.e.* immersed in a bath of hot dilute hydrochloric or sulphuric acid until, by examination, it is found that all surface oxide is dissolved. If sulphuric acid is used it is then washed in water since presence of this acid or any of its salts prevents the adherence of the coating metals. After this washing, or immediately after pickling if hydrochloric acid is used, the article is immersed in a flux consisting of a solution of zinc chloride (frequently containing free hydrochloric acid) made by neutralising concentrated hydrochloric acid by means of metallic zinc. It is necessary to have a thin deposit of this flux on every part of the surface. It is then plunged below the surface of a molten mixture of lead and tin, containing from 35 per cent. to 90 per cent. of lead, held

in an iron vessel heated from below. Excess of metal is removed from the surface of the article, after taking it from the bath, while still in a molten state, by wiping with a pad of tow or shaking or by some equivalent operation. If the article is made of copper or brass or tin plate the preliminary cleaning by means of acid (pickling) is not necessary and it is at once dipped in flux and plunged into the molten metal.

Evidence of Lead poisoning.

Dr Legge has collected statistics relating to all cases of lead poisoning reported to the Factory Department during the eight years 1899 to 1906, as occurring in processes of tinning. These are given in the following tables¹.

TABLE I¹.

Showing the number of cases of lead poisoning reported in the two four-yearly periods 1899 to 1902 and 1903 to 1906 as occurring in tinning processes.

	1899 to 1902			1903 to 1906		
	Males	Females	Total	Males	Females	Total
Tinning of Holloware	15	14	29	13	22	35
Iron Drums and Kegs	11	—	11	24	—	24
Harness Furniture	8	—	8	7	—	7

This table he has supplemented by one showing the symptoms observed and the severity of the attacks, side by side with similar details relating to all the attacks of lead poisoning reported to the Factory Department during the years 1901 to 1904 inclusive, with the object of comparing the severity of the illness with that observed in lead industries taken as a whole.

In commenting on Table II Dr Legge says¹—

“In the tinning of holloware the severity of the attacks exceeds that noted in other tinning processes, and greatly exceeds that of the total lead poisoning cases for the years 1901 to 1904. The proportion of slight cases among men (10·7 per cent.) is without example in our experience in any lead industry. Third attacks or chronic plumbism among females engaged in the tinning of holloware is nearly five times as frequent as among females employed in lead industries as a whole. This is the more remarkable when the age of the women workers is borne in mind, the average age of the females attacked being only

¹ *Loc. cit.* p. 1.

18½ years as compared with 29 among a similar number of consecutive cases of plumbism in women in 1903."

TABLE II¹.

Showing details of all cases of lead poisoning reported in the eight years 1899 to 1906 as occurring in tinning processes, compared with similar details of all the cases reported as occurring in lead industries as a whole during the four years 1901 to 1904.

			Tinning of Holloware		Iron Drums and Kegs		Harness Furniture		Total		All Lead Industries 1901—1904	
			Attacks	Per cent.	Attacks	Per cent.	Attacks	Per cent.	Attacks	Per cent.	Attacks	Per cent.
Severity of Symptoms :												
Severe	{M	15	53·6	7	20·0	4	26·7	26	33·3	757	33·5
		{F	11	30·6	—	—	—	—	11	30·6	86	21·8
Moderate	...	{M	10	35·7	8	22·9	5	33·3	23	29·5	520	23·0
		{F	12	33·3	—	—	—	—	12	33·3	102	25·8
Slight	{M	3	10·7	20	57·1	6	40·0	29	37·2	917	40·6
		{F	13	36·1	—	—	—	—	13	36·1	196	49·6
Not stated	...	{M	—	—	—	—	—	—	—	—	64	2·8
		{F	—	—	—	—	—	—	—	—	11	2·8
Number of Attack :												
First	{M	16	57·1	24	68·6	4	26·7	44	56·4	1451	64·3
		{F	16	44·4	—	—	—	—	16	44·4	300	75·9
Second	{M	8	28·6	5	14·3	4	26·7	17	21·8	348	15·4
		{F	12	33·3	—	—	—	—	12	33·3	62	15·7
Third	{M	4	14·3	3	8·6	7	46·7	14	17·9	361	16·0
		{F	8	22·2	—	—	—	—	8	22·2	18	4·6
Not stated	...	{M	—	—	3	8·6	—	—	3	3·9	98	4·3
		{F	—	—	—	—	—	—	—	—	15	3·8
Signs in Symptoms :												
Gastric	{M	22	78·6	29	82·9	9	60·0	60	76·9	1845	80·8
		{F	35	97·2	—	—	—	—	35	97·2	317	80·3
Paralytic	{M	12	42·9	7	20·0	6	40·0	25	32·1	479	21·2
		{F	7	16·6	—	—	—	—	7	19·4	61	15·4
Encephalopathic	...	{M	1	3·6	1	2·9	—	—	2	2·6	88	3·9
		{F	1	2·8	—	—	—	—	1	2·8	25	6·5
Rheumatic	...	{M	5	17·9	3	8·6	—	—	8	10·3	241	10·7
		{F	1	2·8	—	—	—	—	1	2·8	48	12·2

Dr Legge also examined a number of workers in tinning processes in 1901 and in 1906 and he gives the results in the following table, in which he also includes, for purposes of comparison, the results of examination of workers in other lead industries.

¹ *Loc. cit.* p. 1.

TABLE III¹.

Showing results of medical examination of workers in tinning and other lead processes. All the workers in tinning processes in sixteen factories were examined.

Occupation	Number examined		Blue Line				Anæmia				Wrist weakness			
	M	F	M	Per cent.	F	Per cent.	M	Per cent.	F	Per cent.	M	Per cent.	F	Per cent.
Tinning processes :														
Holloware (Tinnery) 1906...	28	26	13	46·4	19	73·0	6	21·4	8	30·8	4	14·3	4	15·4
Holloware (Tinnery) 1901...	43	25	18	41·9	13	52·0	15	34·9	8	32·0	4	9·3	1	4·0
Harness Furniture ...	25	—	13	52·0	—	—	14	56·0	—	—	4	16·0	—	—
Metal drums & casks (Tinnery) ...	20	—	14	70·0	—	—	8	40·0	—	—	3	15·0	—	—
Total tinnery in holloware, harness furniture & metal casks (1901) ...	98	—	45	45·9	—	—	37	37·8	—	—	11	11·2	—	—
Enamelling processes :														
Baths ...	83	—	40	48·2	—	—	25	30·1	—	—	7	8·4	—	—
Copper letters, etc. ...	12	103	3	—	43	41·8	1	—	45	43·7	—	—	5	4·8
Sheet iron—														
(1) With use of lead														
Factory 1 ...	2	41	1	—	18	43·9	1	—	19	46·3	—	—	1	2·4
Factory 2 ...	11	44	4	—	16	36·3	1	—	10	22·5	—	—	—	—
(2) Without use of lead...	—	80	—	—	2	2·5	—	—	31	38·8	—	—	—	—
Jewellery & watch dials ...	44	31	4	9·1	6	19·4	2	4·5	4	12·9	—	—	—	—
Yarn dyeing with chromate of lead ...														
... ..	45	139	9	20·0	20	14·4	2	4·4	22	15·8	—	—	1	0·7
Paints & colours ...	251	—	103	41·0	—	—	88	35·0	—	—	9	3·6	—	—

In the particular factory where the experiments were carried out one case of lead poisoning was reported in 1903 in which the symptoms were recorded as long standing paralysis, and a second in which they were colic, headache, dimness of vision, and general weakness. The former patient died a few months later from pulmonary tuberculosis. Dr Legge examined the three men at work during the experiments. Two of them doing the greater part of the common tinning were markedly anæmic and had a blue line, and the third, who was only irregularly at work in the tinning workshop and then chiefly at bright tinning (pure tin), showed a trace of a blue line only. One of the former was reported as suffering from lead poisoning early in 1907.

¹ *Loc. cit.* p. 1.

Preliminary investigation.

It appeared possible from a preliminary examination of the process of tinning as carried on in works where cases of lead poisoning had occurred that the illness might be caused by—(a) Inhalation of vapour of lead chloride, produced by interaction of the hydrochloric acid and zinc chloride necessarily present on the surface of the article to be tinned at the moment it is brought in contact with the molten metal, with the lead of the tinning mixture, and vapourised at the temperature of the molten metal. (b) By inhalation of minute particles of metal or lead chloride in the solid state caused to enter the air by the mechanical action of the rapidly escaping vapour produced below the surface of the molten metal when the article to be tinned is thrust into the metal bath. (c) By inhalation of dust containing lead or compounds of lead caused to float in the air by sweeping the sides of the bath, etc. (d) By want of cleanliness on the part of workers. A number of experiments in which air was aspirated from a point over the molten metal during tinning, and bubbled through water, were carried out, and the washing liquid subsequently examined qualitatively. In all cases minute quantities of lead were found to be present in the form of chloride. Zinc chloride and free hydrochloric acid were also invariably found and in some cases a compound of tin was present. The washing liquid remained quite clear except in one case where the experiment was of a more lengthy nature. In this a white precipitate formed and proved to be pure oxide of tin. The lead was always found in solution.

Large deposits of dust were invariably observed on every ledge in tinning workshops, and samples of this were taken from various points and qualitatively examined. Those samples taken from points near a tinning bath contained considerable quantities of lead chloride and also some lead insoluble in water. Zinc chloride, copper chloride and a compound of tin were also observed. Samples collected from points remote from a tinning bath appeared to contain small quantities only of lead chloride, but lead insoluble in water was always noted. Large quantities of fibre apparently similar to the tow used in wiping excess of metal from the tinned article were invariably observed in the samples of dust from whatever point collected. It therefore appeared that the dissemination of particles of fibre carrying small quantities of lead and lead chloride, rubbed off the hot tinned surface during wiping, might be a further source of lead poisoning. As a result of this preliminary investigation more accurate experiments were devised with

a view to showing the extent and source of contamination of the atmosphere by compounds of lead, etc.

Accuracy of experiments and results.

The difficulties in the way of arriving at any accurate knowledge of the quantities of foreign substances present in the atmosphere of a tinning workshop were immense. The preliminary investigation indicated that the actual amounts of any deleterious substance present at any given time are not only small, but also that they vary enormously in different parts of the workshop and also in the same position at different times according to the nature of the work being carried on. It appeared hopeless to attempt to obtain accurate results by means of analyses of air, and moreover from the point of view of the enquiry such results would have very little value since on account of the variations in the state of the atmosphere they could not be applied with any certainty to show the probable effect on the health of the workers. The most promising line of investigation appeared to be to ascertain the quantities of various deleterious substances present in a large volume of air taken continuously from a given point during a considerable period of time, and to compare the results with the average time spent and the average amounts of air inhaled by a worker at a particular operation—in other words to ascertain what quantities of deleterious substances a worker would on the average inhale in a given time. Under such conditions there appeared to be no possibility of dealing with scientifically accurate volumes of air reduced to normal temperature and pressure, since not only were the volumes to be examined very large in the total but it was necessary to carry on some of the experiments from day to day for several days, during which the temperature and pressure would vary continuously. Hence it is not claimed that the experiments and their results show accurately the composition of the air of a tinning workshop at a given time. It is however claimed that they show the approximate amounts of deleterious substances inhaled by workers during given periods of time, and that they may be used as a basis for calculation of the effects on their health when exposed to such conditions as obtain in tinning workshops.

Quantities of deleterious substances inhaled by workers.

This section is inserted here for convenience, but the quantities of deleterious substances inhaled by workers are calculated from experimental data which are given in subsequent sections dealing with the experiments. The substances experimentally found to exist in the atmosphere of a tinning workshop consist of hydrochloric acid and chlorides of lead, zinc, iron and copper in the state of vapour, or closely approaching that state, with metallic tin and lead carried mechanically on fibre from the tow used in wiping. Hydrochloric acid is however only observed in quantity in the immediate neighbourhood of the baths though it probably collects also near the roof. The quantity of any of these substances present in the air at a given time is very small. Thus it would appear that, in the neighbourhood of the tinning baths and wiping stands, the quantity of lead chloride ranges from approximately two parts to sixty parts per ten million parts by weight of air. Dr Legge however points out in quoting medical authorities that it is not so much the quantity of lead taken in any case which determines the symptoms as its continued introduction, and also that for the production of chronic plumbism long continued absorption of small quantities of lead is necessary¹. The effect on the health of workers of long continued absorption of small quantities of such compounds as hydrochloric acid and chlorides of zinc, tin, copper and iron must be left to medical authorities, but apparently it must at least result in impaired vitality. In the following table an attempt (based on the experimental data subsequently given) has been made to estimate the quantities of various deleterious substances inhaled by workers per day and per year. The method of arriving at the results given was as follows: the actual average time a tinner, who prepares his own work, spends in the tinning workshop during tinning and wiping, as distinguished from the time spent outside in preparing his work (*i.e.* pickling, etc.), was ascertained by observation extending over several weeks. It worked out at slightly over $5\frac{1}{2}$ hours per day. Taking the average quantity of air inhaled by a man as 18 cubic feet per hour, a day's work would involve his breathing about 100 cubic feet of the air of the tinning workshop during the times when fumes are being produced. The same data are available whether a worker does tinning or wiping since these operations are interdependent. Hence it is possible to calculate the results shown in the table.

¹ *Loc. cit.* p. 1.

TABLE IV.

Showing the quantities of deleterious substances inhaled by workers in a tinning workshop.

The following assumptions are made :

Average volume of air inhaled at each inspiration = 30·5 cubic inches¹.

Average number of inspirations per minute = 17¹.

Average time spent in tinning, wiping, etc. per day = 5 hours, 34 minutes.

Average number of days worked per week = 5.

Average number of weeks worked per year = 50 weeks = 250 days.

N.B. The figures in brackets are the quantities of the various compounds expressed in grams.

Worker affected		Distinctive no. of exp. on which conclusion is based	Quantities expressed in grams of deleterious substances inhaled by workers in a tinning workshop per day and per year							
			Soluble in water					Insoluble in water		
			Chlorides of				Hydro-chloric acid	Oxide of tin	Metallic	
			Lead	Zinc	Iron	Copper			Tin	Lead
Tinner using open tinning bath	Per day	5	0·0143 (0·221)	0·0607 (0·937)	0·0250 (0·386)	Trace	0·3897 (6·014)	0·0070 (0·011)	—	—
	Per year	5	3·575 (55·169)	15·175 (234·181)	6·250 (96·45)	Small amt.	97·425 (1503·463)	1·750 (27·006)	—	—
Tinner using bath covered by hood & having fumes exhausted by draught off furnace chimney	Per day	6	0·0024 (0·037)	0·0155 (0·239)	0·0052 (0·080)	Trace	0·0090 (0·014)	0·0018 (0·028)	—	—
	Per year	6	0·600 (9·259)	3·875 (59·799)	1·300 (20·062)	Small amt.	2·250 (34·722)	0·450 (6·944)	—	—
Tinner using open bath and wearing respirator	Per day	7	0·0034 (0·052)	0·0075 (0·116)	0·0069 (0·106)	—	0·2879 (4·443)	Trace	—	—
	Per year	7	0·850 (13·117)	1·875 (28·935)	1·725 (26·620)	—	71·975 (1110·718)	Small amt.	—	—
Wiper	Per day	8	0·0191 (0·294)	0·0125 (0·193)	0·0091 (0·222)	—	Nil	0·0068 (0·105)	0·0259 (0·400)	0·021 (0·320)
	Per year	8	4·775 (73·688)	3·125 (48·125)	2·275 (35·108)	—	Nil	1·700 (26·234)	6·475 (99·922)	5·27 (81·40)

For reasons stated in subsequent sections the quantities of deleterious substances given in the table as being inhaled by workers at an open bath, with or without a respirator, must be taken as the minimum. Actually they would probably inhale somewhat larger quantities. Consideration of this table shows that of all the persons working in a tinning workshop those doing wiping are exposed to most

¹ *Hygiene and Public Health*, by Parkes and Kenwood, p. 211.

danger from lead chloride in the form of vapour, or in a state closely approaching that of vapour, though a tinner using an open bath (which is not very general) would be exposed to almost as much risk. The results are exceedingly interesting and fully support Dr Legge and the medical authorities he quotes in the statement that long continued absorption of small quantities of lead is a factor of much more importance in causing lead poisoning than the amount of the dose. There can be no doubt, as will be seen later, that vapour of lead chloride is being continually given off from the tinned article for some time after its removal from the bath, probably in decreasing quantity till the coating is set and hard.

Experimental.

The whole of the laborious quantitative analyses were carried out by Dr Thorpe, C.B., F.R.S., in the Government Laboratory, and we desire to take this opportunity of publicly acknowledging our indebtedness to him and to Mr E. Grant Hooper, also of the Government Laboratory, for their able assistance in this respect.

The investigation naturally divided itself into three parts—(a) A series of laboratory experiments under conditions allowing of control of any fumes produced. (b) A series of workshop experiments. (c) The collection of samples of dust from selected points and their subsequent examination in order to obtain some knowledge of the distribution of the deleterious substances in the atmosphere.

After a large number of preliminary experiments the following general method of examination of the atmosphere was adopted. An aspirator consisting of an ordinary filter pump attached to a water tap was connected by red rubber pressure tubing to the outlet pipe of a measuring instrument. This was a specially made wet gas meter, of the type used in research work on coal gas, designed to accurately measure volumes of gas, but suitably altered for these experiments. It was fitted with devices for maintaining the water at the same level as that of calibration and also with a glass observation chamber in which was fitted a thermometer. The inlet pipe of this instrument was connected by pressure tubing to an absorption apparatus consisting of two tall glass vessels each containing about 180 c.c. of distilled water and connected with each other in such a way that, on allowing water to pass through the aspirator, air was bubbled through the water in each vessel in succession and was then passed through the meter in order to

measure its volume. In most cases it was found that the absorption apparatus acted as a very efficient filter, the air being freed from all foreign substances during passage through it. In order to ensure its isolation and to prevent accidental introduction of impurities large glass bulbs were fitted to the inlet tube of one and the outlet tube of the other vessel of the absorption apparatus. To the inlet bulb was attached a gun metal funnel inside of which were fitted two removable iron gauze diaphragms three inches apart, in order to further ensure isolation of the absorption apparatus and to prevent small splashes of metal finding their way into it. In some experiments the space between the diaphragms was filled with closely packed glass wool, the object of its insertion being to differentiate between fumes and dust, the preliminary experiments having shown that while this material effectively filtered dust from the air it was not capable of entirely preventing passage of vapour. In the workshop experiments the whole of the absorption apparatus was clamped to a retort stand and it could thus be placed with the mouth of the funnel at any point from which it was desired to aspirate air, the rubber tubing connecting it with the meter having a length of about forty feet. The air from the selected point was thus passed through the gauze diaphragms and glass wool filter in the funnel, through the inlet guard bulb and then through the water in each vessel in turn. The foreign substances remained either in the glass wool or in the water filters while the air passed on to the meter. The object of having a second washing vessel was so that it might act as a control on the first and afford some guide as to whether filtration was efficient. The apparatus was watched during the whole time of experiment and observations of temperature and volume aspirated were made every fifteen minutes.

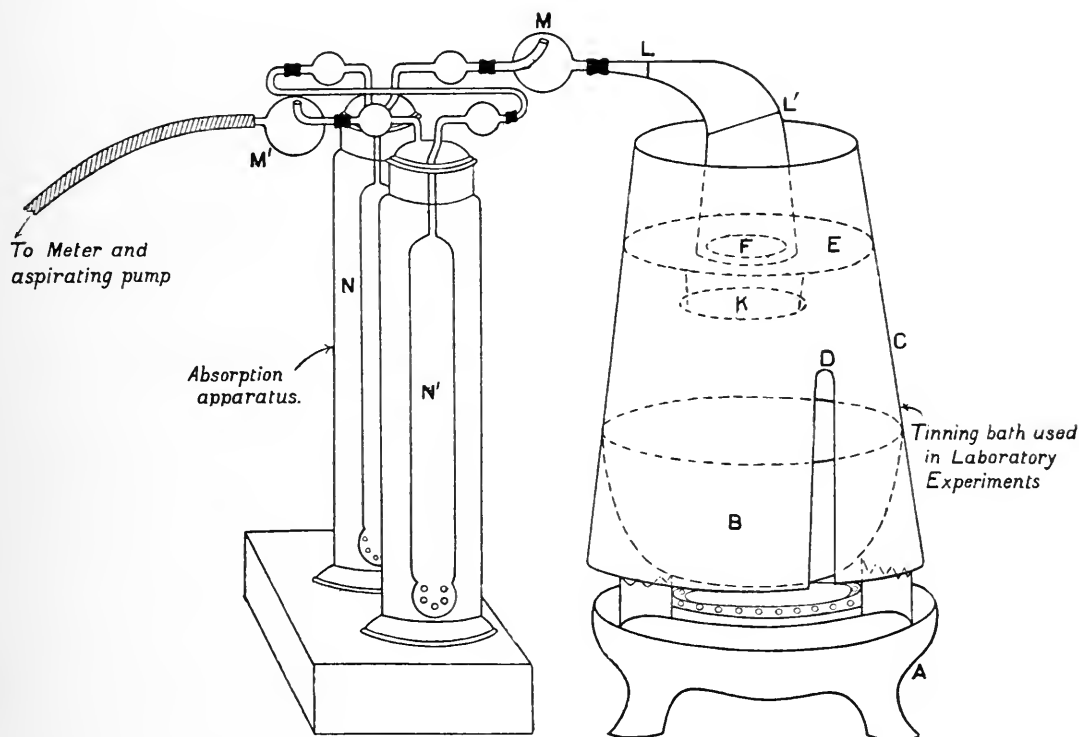
Sketch of apparatus used.

The absorption apparatus is shown on the left and the tinning bath used in the laboratory experiments on the right. The sketch shows the apparatus as fitted up for the laboratory experiments.

Laboratory experiments.

In these experiments it was necessary to have any fumes produced under control while reproducing as far as possible the conditions of tinning. After many attempts, which showed considerable danger of injury from explosions in the molten metal, the following method was

devised. A thin iron bowl capable of holding about 1200 grams of molten metal was supported on a Fletcher gas burner and completely covered by an iron cowl in the form of a frustum of a cone having at one side a vertical slot about $\frac{3}{4}$ in. wide extending about $2\frac{1}{2}$ ins. above the edge of the bowl. Inside the cowl and about $3\frac{1}{2}$ ins. above the surface of the molten metal an iron disc was fitted, having at its centre a circular orifice slightly smaller than the mouth of the funnel of



- A. A gas burner for heating B which is a thin iron bowl, capable of holding about 1200 grams of molten tinning metal, resting on supports on the burner A.
- C. An iron cowl fitting closely over B and having a slot D about $\frac{3}{4}$ in. wide cut in it to admit of introduction of articles to be tinned.
- E. A disc of iron, fastened inside C, having a circular hole F cut at its centre and a second disc K attached to it below and slightly larger than the hole F. Its object was, while allowing of passage of fumes, to prevent molten metal from splashing upwards.
- LL'. A gun metal funnel forming part of, and attached by rubber tubing to, the absorption apparatus. In an experiment the absorption apparatus was so placed as to bring the mouth of the funnel to the point from which it was desired to aspirate air. Thus in the illustration it is shown fitted over the hole F in the disc E above the tinning bath used in the laboratory experiments. At L and L' iron gauze diaphragms were fastened inside the funnel and the space of 3 inches between them was in some experiments filled with closely packed glass wool.
- M and M'. Large glass guard bulbs attached by rubber tubing to the inlet and outlet tubes respectively of the washing vessels.
- N and N'. First and second washing vessel respectively, each containing about 180 c.c. of distilled water.

the absorption apparatus, and about an inch below this another iron disc was suspended, larger than the orifice but not so large as the first disc, but completely preventing direct splashing of metal through the orifice. The funnel of the absorption apparatus was fixed in contact with the upper disc so as to completely cover the orifice. The object of the discs as arranged was to confine the atmosphere above the molten metal and allow its passage into the absorption apparatus while preventing splashing of metal into the funnel.

Experiments I and II. The objects of the first two experiments were (a) To show the effect of the violent escape of vapour, produced below the surface of molten metal, in causing contamination of air, (b) To ascertain the nature of these contaminating substances, and (c) To ascertain whether they can be filtered from air by a material capable of use in a respirator. As the effect of violently escaping vapour on molten metal is, from one point of view at least, purely mechanical, it was necessary in these experiments to avoid any complicating chemical action. Hence no hydrochloric acid or zinc chloride flux could be used. At the same time it was necessary to reproduce the process of tinning as performed in a workshop as far as possible. A tinning metal consisting of a mixture of half lead and half tin was made by weighing out the pure metals separately, melting them together and removing all dross. The metal was then placed in the iron bowl described, and the apparatus fitted up. A number of pieces of iron about one inch square were bent so as to afford a holding surface for liquid and each of these was held in a pair of slender crucible tongs, dipped in distilled water and immediately introduced through the slot in the cowl covering the bowl and plunged into the molten metal. Aspiration was commenced after the introduction of the third piece of iron and was continued at the rate of about four cubic feet per hour. The rate at which the wet pieces of iron were introduced was about eighty per hour. Alarming and violent explosions occurred as they came in contact with the molten metal and it was at once noted that these were much more intense than anything which occurs in actual tinning. It was quickly apparent that contamination of the atmosphere can be caused in this way since a grey sediment began to collect in the absorption apparatus. The space between the gauze diaphragms was not, in the first experiment, filled with glass wool and at the close of this experiment a considerable deposit of a heavy extremely finely divided greyish black powder was found in both the inlet guard bulb and in the first washing vessel but not in the second. A deposit of the

same dust was noted on surfaces near the apparatus and on the surface of the molten metal. The contents of the inlet guard bulb and the first washing vessel were examined separately. In the former the substance deposited was found to consist principally of metallic tin and lead oxide with a smaller proportion of tin oxide. In the washing vessel the substance deposited was found to consist of tin oxide, metallic tin, and lead oxide in nearly equal proportions. In both samples the ratio of tin to lead was much greater than in the metal from which they were derived, showing that the tin is more readily caused to enter the atmosphere than lead.

In the second experiment the space of three inches between the gauze diaphragms in the funnel of the absorption apparatus was filled with closely packed glass wool, but in other respects the method followed was exactly the same as in Exp. I. Cotton wool would no doubt have been a better filtering material than glass wool but preliminary experiments had shown that the fumes produced in tinning quickly condensed in cotton wool and prevented passage of air, and, since it was desired to make the experiments comparable as far as possible, glass wool was therefore used in all those experiments where an additional filter was required. The phenomena noted were similar to those observed in the first experiment with the significant exception that no deposit of dust could be observed in either the guard bulb or the washing vessel, though the volume of air aspirated was more than double and the length of the experiment twice that of the first experiment. At the end the glass wool composing the lower eighth of an inch of the filter was found to contain a considerable quantity of the finely divided greyish black powder and for a space of half an inch the filter was slightly discoloured. The remainder was however quite clean and bright indicating that efficient filtration of dust from the air had been obtained. The liquid condensed in the inlet guard bulb and the contents of the first washing vessel were washed into one sample bottle and examined together. The analytical report however stated that no lead, tin or other solid substance was present. It was in fact still pure distilled water. The analysis of the black powder collected in the glass wool filter showed it to be of the same approximate composition as that of the two samples, collected in the first experiment, combined. The results of the two experiments are summarised in the following table.

The results of the two experiments clearly show that the mechanical action of escaping vapour produced below the surface of molten metal may be a serious factor in causing contamination of the atmosphere by

TABLE V.

Showing the quantities of deleterious substances collected in the absorption apparatus in experiments showing the effect of the mechanical action of escaping vapour, produced below the surface of molten metal, in causing contamination of the air.

The percentage composition of the foreign matters present in the air is shown by the figures in brackets below those expressing the quantities. In Experiment I the figures given are the combined results of examination of two samples collected separately. In Experiment II the percentage composition is stated as the result of examination of dust collected in the glass wool filter.

Exp. in which collected	Volume in cubic feet aspirated	Quantities expressed in grams of various deleterious substances found deposited in washing vessels								Ratio $\frac{\text{Tin}}{\text{Lead}}$	
		In solution			In suspension					In sample	In original tinning mixture
		Calculated as metal		Sulphuric acid	Metallic tin	Tin oxide	Metallic lead	Lead oxide	Iron oxide		
		Lead	Tin								
I	10.0000	0.0014 (0.28)	0.0111 (2.24)	Trace (Trace)	0.2066 (41.64)	0.0725 (14.61)	Nil (Nil)	0.2008 (40.48)	0.0037 (0.75)	$\frac{1.46}{1}$	$\frac{1}{1}$
II	20.0117	Nil —	Nil —	Nil —	Nil (45.30)	Nil (14.20)	Nil (Nil)	Nil (39.90)	Nil (0.60)	$\frac{1.5}{1}$	$\frac{1}{1}$

lead and tin either in the form of dust or fume or both, the lead being in the form of oxide and the tin in the form of both metal and oxide. They also show that whatever the origin of the contaminating substances whether as dust or fume or spray their inhalation can be readily prevented by use of a respirator. A further conclusion and one of great importance in following experiments is that glass wool arranged in the manner described is perfectly efficient as a filter for removing dust from the air drawn through it.

Experiments III and IV. These experiments were devised to afford information as to (a) The nature of the emanations from the tinning bath, (b) Whether the deleterious substances exist in the air as vapour or dust and as to the possibility of filtering them from the air by material capable of use in a respirator. The apparatus used and the general method followed were the same as in the first two experiments but the processes of tinning were exactly reproduced. A solution of zinc chloride was prepared by adding excess of zinc to pure concentrated hydrochloric acid and the pieces of iron were immersed in warm dilute hydrochloric acid till free from all surface oxide. They were then drained free of excess of acid, dipped singly in the zinc chloride solution (flux) and introduced into the molten metal through the slot in the cowl

over the metal bath, the rate of tinning being about 60 pieces per hour. On removal from the molten metal they were found to be properly tinned. In Exp. III the space between the gauze diaphragms of the funnel of the absorption apparatus was empty but in Exp. IV this was filled with closely packed glass wool. In both experiments immediately on commencing tinning and aspiration the whole apparatus became filled with dense white fumes, and in about fifteen or twenty minutes a faint milkiness was observed in the liquids of the washing vessels and this gradually increased till a considerable quantity of a creamy white precipitate was produced in each vessel, though that in the first was greater in quantity than in the second. Exp. IV had to be interrupted several times and the apparatus dismantled in order to renew the glass wool filter, the condensation of fumes in it causing complete cessation of passage of air. In no other respect was any outward difference to be observed in the phenomena noted in the two experiments. On ceasing aspiration a bluish vapour was observed issuing from the slot in the cowl and aspiration was therefore continued for some time after tinning had ceased, but in Exp. IV blocking of the glass wool filter was then very rapid, the lower part becoming completely cemented up by a white highly deliquescent substance. No dust was observed as in the first pair of experiments, but at the conclusion of each experiment it was noted that a yellowish grey soft pasty semi-fused mass of some substance had collected on the surface of the molten metal (this is to be observed also in workshop tinning baths) which, on cooling, solidified into a hard greyish resinous looking substance. The blue vapour was seen to rise from it when hot. On the inside of the cowl and funnel there was a deposit of a white intensely deliquescent substance and round the gauze diaphragms and the lower edges of the funnel there were fringes of white needle shaped crystals which were not deliquescent. All these phenomena appear to point to the production of true vapour during tinning. The contents of each washing vessel were examined separately and it was found in both experiments in each case that the condensed fumes consisted of lead chloride, zinc chloride, iron chloride and hydrochloric acid in solution while the creamy white precipitate was oxide of tin. In Exp. IV the amount of lead chloride was about two-thirds, that of zinc chloride five-sixths, that of hydrochloric acid about double and that of tin oxide about one-third more than that found in the absorption apparatus in Exp. III, though the air in the former case had to pass through three inches of closely packed glass wool before reaching the washing vessels, while its passage was quite uninterrupted

in the latter case. No examination was made of the glass wool filter. A striking difference was observed in the behaviour of the molten metal when the pieces of iron previously dipped in zinc chloride flux were plunged below its surface as compared with the same operation with pieces of iron previously dipped in water. In the latter case explosions, always violent and frequently alarmingly so, occurred, while in the former there was a mild bubbling only of the metal. Hence though the mechanical action of escaping vapour in causing contamination of the atmosphere cannot be ignored it is to be concluded that the effect in actual tinning is not nearly so pronounced as might be expected from the results of Exps. I and II. The pasty substance collecting on the surface of the molten metal must also tend to prevent the production of dust. It is striking that while in Exp. II it was conclusively shown that all the substances caused to enter the air by the purely mechanical action of escaping vapour could quite easily be filtered from the air by means of a mechanical filter, Exp. IV shows that the substances caused to contaminate the atmosphere in actual tinning cannot be separated in this way. It is noteworthy also that tin oxide should be found in solid form in the washing vessels in Exp. IV. It could not pass the glass wool filter in this state and it is equally impossible that it could exist in the air as vapour of tin oxide. One is therefore driven to the conclusion that it must be caused to enter the atmosphere as the vapour of some compound of tin capable of decomposition by water—probably as tin chloride. The results of Exps. III and IV are given in the following table.

The conclusions drawn from the two experiments are—(a) That the processes of tinning cause the atmosphere to become impregnated with hydrochloric acid and chlorides of lead, zinc, iron and probably tin. (b) Use of a respirator by workers is impossible by reason of the tendency of the chlorides to condense and block up the filtering material. (c) Even if possible of use inhalation of substances caused to contaminate the air by tinning cannot be prevented by use of a respirator. (d) The mechanical action of escaping vapour is not nearly so important a factor in causing air contamination as the chemical action of the materials (acid and flux) used in tinning on the tinning metal and subsequent vapourisation of the products of this action.

A comparison of the results of Experiment I with those of Experiments III and IV brings out a striking point. The experiments are strictly comparable, the only radical difference being that in I distilled water only and in III and IV hydrochloric acid and zinc chloride

TABLE VI.

Showing the quantities of deleterious substances condensed in the absorption apparatus after having been caused to enter the air by the process of tinning as conducted in laboratory Experiments III and IV.

The percentage composition of the foreign matters thus filtered from the atmosphere is shown by the figures in brackets given below those showing the quantities of the various deleterious substances.

Volume in cubic feet of air aspirated	Quantities expressed in grams of deleterious substances condensed in absorption apparatus in each experiment									Ratio Tin Lead	
	In solution					In suspension				In samples	In original tinning mixture
	Chlorides of				Hydrochloric acid	Oxides of					
	Lead	Zinc	Iron	Copper		Tin	Lead	Iron	Zinc		
14.0000	0.0384 (3.37)	0.6620 (58.17)	0.0041 (0.36)	— —	0.3240 (28.47)	0.1082 (9.51)	0.0010 (0.09)	0.0003 (0.02)	Trace	$\frac{2.9}{1}$	$\frac{1}{1}$
14.5800	0.0233 (1.52)	0.5530 (36.14)	0.0021 (0.14)	— —	0.8150 (53.26)	0.1361 (8.89)	0.0006 (0.04)	0.0002 (0.01)	Trace	$\frac{5.99}{1}$	$\frac{1}{1}$

solution were brought into contact with molten metal. In Experiment I it is shown that 10 c. ft. of air contained 210.2 milligrams of lead oxide while in the third experiment 14.0 c. ft. are shown to contain only 38.4 milligrams of lead chloride and 1 milligram of lead oxide. Further it is shown by Experiment II that all the products of mechanical action of escaping vapour can be filtered from air by means of the glass wool filter, while in Experiment IV after passage through an exactly similar filter 14.58 c. ft. of air are shown to contain 23.3 milligrams of lead chloride and 0.6 milligram of lead oxide (very probably this is really lead chloride mechanically held entangled in the tin oxide precipitate). The difference in the amounts of lead chloride in the air shown by Experiments III and IV, *i.e.* 15.1 milligrams, must be ascribed to the use of the glass wool filter, and any substances caused to enter the air in these experiments by the mechanical action of escaping vapour would certainly be arrested by it. Hence the quantity of lead oxide caused to contaminate the air in this way must be considerably less than 15.1 milligrams, since there can be no reasonable doubt that some of the lead chloride vapour also condensed in the filter and therefore formed part of the difference of 15.1 milligrams shown. The result shows that the mechanical action of vapour in producing air contamination in tinning is small as compared with that of chemical action.

¹ In this experiment the air before passing through the liquid in the absorption apparatus was drawn through 3 inches of closely packed glass wool.

Workshop experiments.

In the workshop experiments the method of examination of the air was exactly the same as that described in Experiments I to IV, the absorption apparatus being placed in such a position as to bring the mouth of the funnel to the point from which it was desired to aspirate air.

Experiment V. The object of this experiment was—(a) To obtain information as to the amount and nature of the contamination of air caused by actual tinning, *i.e.* dipping. (b) To afford a basis for calculation of the danger to and effect on the health of a tinner using an open bath. It was carried out in connection with a tinning bath provided with a hood completely enclosing the metal bath on three sides and provided with exhaust as good as it is possible to obtain by means of an opening from the hood into the furnace chimney. The day was bright and sunny and fumes were removed fairly quickly, the atmosphere being free from the choking property usually associated with tinning workshops. The metal was new and was bright and clean and aspiration was continued for some time when no tinning was in progress. It is to be expected therefore that the results are lower than would on the average be obtained if the tinning bath were quite open, and that they show the minimum danger to which a tinner using an open bath would be exposed. The space between the gauze diaphragms in the funnel of the absorption apparatus was not filled with glass wool and the air over the molten metal was therefore drawn directly through the distilled water in the washing vessels after passage only through the two gauze diaphragms and the guard bulb. The absorption apparatus was placed in such a position inside the hood over the tinning bath as to bring the mouth of the funnel nearly over the centre of and about ten inches above the molten metal. The contents of each washing vessel were separately examined and the results of analysis showed the atmosphere over the bath to be impregnated with hydrochloric acid and chlorides of lead, tin, zinc and iron, and that a tinner using an open bath must inhale considerable quantities of these substances.

Experiment VI. This experiment was devised for the purpose of showing—(a) The condition of the atmosphere at a moderate distance from the metal bath. (b) The danger to the health of a tinner working at a bath provided with a hood and exhaust by connection with the furnace chimney. It was carried out in connection with the same bath as Experiment V, but the days were less favourable to the

escape of fumes and the atmosphere during parts of the experiment was bad. The hood completely enclosed the bath except at the front where the tinner works and this was partly closed by an iron apron suspended from the top of the hood. The absorption apparatus was suspended in such a position as to bring the mouth of the funnel exactly on a level with the tinner's nose (indeed he had to exercise care to avoid knocking it) and about eight inches outside and away from the apron partly closing the front of the hood, and about one foot above its lower edge. In this way only the air actually possible of inhalation by the worker was drawn through the apparatus and direct access of fumes from the molten metal bath was impossible. Blue vapours could occasionally be seen rising from the articles as they were removed from the tinning bath to the wiping stand and also issuing from below the lower edge of the apron covering part of the front of the hood. The experiment was continued from day to day till 100 c. ft. of air had passed through the absorption apparatus and occupied five days in all. It had previously been noted that fumes containing lead chloride became deposited on the outside of the apparatus and in all the experiments great care was taken to prevent contamination of the liquids in the washing vessels from this source, the whole apparatus being carefully wiped and the contents of each vessel transferred to marked stoppered bottles without opening the washing vessel at the conclusion of each day's work and similarly replaced at the beginning of the next. The apparatus was watched during the whole time so as to insure no accidental interference from the workers. The contents of each vessel were examined separately and the results showed that the air contained hydrochloric acid and chlorides of lead, zinc, tin and iron of which substances a tinner, even when using a bath provided with a hood connected to the furnace chimney, must inhale considerable quantities. It seems probable however that a considerable proportion of the chlorides of lead, etc. emanate not from the bath directly but from the tinned article as it is removed from the bath to the wiping stand. (See Experiment VIII.) The space between the diaphragms of the funnel of the absorption apparatus was empty in this experiment.

Experiment VII. This experiment is complementary to No. V and it is also comparable with Experiment IV. Its objects were—(a) To show the value of a respirator to a tinner. (b) To afford a basis for calculation of the danger to health of a tinner wearing a respirator but working at an open bath. (c) To afford some means of comparison

between the respective values of a respirator, and a hood with connection to the furnace chimney, to a tinner. The experiment was carried out in connection with the same bath as Nos. V and VI, the absorption apparatus being fixed in such a position inside the hood as to bring the mouth of the funnel about 3 ft. 10 ins. above the level of the molten metal, considerably to one side of the bath and just inside the apron partly closing the front of the hood. In this position the point from which air was aspirated was on the side of the bath opposite to the exhaust into the furnace chimney and was somewhat above breathing level. As in Experiment V, therefore, the results are probably lower than the average which would be obtained on an open bath, since the fumes were drawn away from the apparatus. Similarly the average amounts of deleterious substances inhaled by a tinner using an open bath would probably be considerably higher than those given as a result of this experiment. Hence it can only be taken as showing the minimum danger to health of a tinner wearing a respirator and using such a bath. It was however desired in this experiment to make the conditions distinctly unfavourable to the passage of the vapours through a glass wool filter. The space of three inches between the gauze diaphragms in the funnel of the absorption apparatus was filled with closely packed glass wool. The experiment was continued from day to day for five days and precautions were taken to avoid accidental contamination of the liquids in the washing vessel. As in Experiment IV difficulty was experienced in maintaining a passage for air through the glass wool filter, the fumes condensing in it and blocking it up. The contents of each washing vessel were separately examined and the results showed the air, even after passage through the glass wool, to contain hydrochloric acid and chlorides of lead, zinc, tin and iron. Experiment II showed that all dust could easily be arrested in the glass wool, and hence one is driven to the conclusion that the chlorides must exist in the air as vapour or in a state very closely approximating to that of vapour, though undoubtedly considerable quantities condense in the filter. The results also clearly show that it is not practicable for a tinner to use a respirator and that even if he could use it he would still inhale appreciable quantities of hydrochloric acid and chlorides of lead, zinc and tin, etc. though probably not to the same extent as a tinner using an open bath and no respirator but to a greater extent than one using a bath with hood and exhaust to the furnace chimney.

Experiment VIII. The object of this experiment was to—
(a) Ascertain the nature and extent of air contamination caused by

the operation of wiping excess of metal, while still in a molten state, from the tinned article. (b) To afford a basis for calculation of the effect of his work on the health of a wiper. The funnel of the absorption apparatus was connected with a large hood, made of thin sheet iron in the form of an inverted bucket, and was so placed that the lower edge of this hood was exactly on a level with the wiper's nose, the kettle being wiped being immediately under the centre of the hood. Aspiration was continued for six days. At the end of the experiment small particles of fibre, obviously derived from the tow used in wiping, could be observed in the first washing vessel but the closest examination could reveal none in the second. Occasionally during the experiment blue vapours could be seen rising from the hot tinned article and passing through the glass tubes of the absorption apparatus. The contents of each washing vessel were examined separately. Considerable quantities of metallic lead and tin, and fibre were found in suspension in the first washing vessel with chlorides of lead, zinc and iron in solution. In the second vessel no fibre nor metallic lead and tin was present though appreciable quantities of chlorides of lead, zinc and iron were found in solution. No hydrochloric acid was found in the free state in either vessel. In Experiment I it was noted that all dust was arrested in the first washing vessel and this is supported by the results of this experiment, all the solid matters being found in the first vessel and probably condensed vapour only provided the deleterious substances found in the second. The fact that particles of fibre are shown to float in the air is extremely important. They are produced during rubbing of the hot tinned surface of the kettle with tow and no doubt they collect minute quantities of metallic lead and tin and also of lead chloride, etc. before they become disseminated in the atmosphere. Indeed probably the whole of the metallic lead and tin and a small portion of the chlorides of lead, zinc, etc. found in the liquids of the absorption apparatus were carried into it by means of the small pieces of fibre. It at once becomes a question as to how much of the soluble lead (lead chloride) existing in the air of a tinning workshop is due to this. It will be seen later from a consideration of the results of examination of samples of dust that this is only small in all probability. The result of the experiment clearly showed that wiping is one of the most dangerous processes of tinning since not only is the wiper compelled to inhale considerable quantities of chlorides of lead, zinc, and iron as vapour, these being larger than those inhaled by a tinner using even an open

bath, but he will also inhale appreciable quantities of fibre carrying metallic lead and tin and chloride of lead.

In the following table the results of the workshop experiments are summarised.

TABLE VII.

Showing the quantities of deleterious substances found in the liquid of the absorption apparatus after passage of air aspirated from different points in a tinning workshop.

The percentage composition of the condensed fumes, etc. is shown by the figures in brackets below those giving the quantities of the various deleterious substances.

Experiment in which collected	Volume in cubic feet aspirated	Quantities, expressed in grams, of deleterious substances condensed in the absorption apparatus in each experiment										Ratio	
		In solution					In suspension					In sample	Tin Lead
		Chlorides of				Hydro-chloric acid	Oxides of				Foreign matters (fibre etc.)		
		Lead	Zinc	Iron	Copper		Tin	Lead	Iron	Zinc			
V	27·2000	0·0039 (2·89)	0·0165 (12·21)	0·0068 (5·03)	Trace	0·1060 (78·46)	0·0019 (1·41)	—	—	—	—	$\frac{0·5}{1}$	1·
VI	100·0300	0·0024 (7·08)	0·0155 (45·72)	0·0052 (15·34)	Trace	0·0090 (26·55)	0·0018 (5·31)	—	—	—	—	$\frac{0·77}{1}$	1·
VII ¹	102·1200	0·0035 (1·13)	0·0077 (2·46)	0·0070 (2·24)	—	0·2940 (94·17)	—	—	—	—	—	$\frac{\text{Trace}}{1}$	1·
Together with a small quantity of tin chloride													
VIII	67·2000	0·0128 (17·00)	0·0084 (11·15)	0·0061 (8·10)	—	Nil	0·0267 (35·46)	0·0153 (20·32)	—	—	0·0060 (7·97)	$\frac{0·89}{1}$	1·
Together with a small quantity of tin chloride													

In the following table certain details as to volume aspirated, work done during the experiments etc. are shown.

¹ In this experiment the air before passing into the absorption apparatus was drawn through three inches of closely packed glass wool.

TABLE VIII.

Showing details of experiments.

Time of aspiration in hours			Duration of exp. in hrs.	Time in hours of aspiration during tinning of		Volume in cubic feet of air aspirated		Total volume of air as- pirated in cubic feet	Temp. in degrees Fah. of air passing through meter		Barometric pressure in inches
When no tinning was in progress	During tinning and wiping	Kettles		Covers	When no tinning was in progress	During tinning and wiping	Maximum		Minimum		
Laboratory experiments :											
—	2 $\frac{27}{60}$	2 $\frac{27}{60}$ (1 day)	—	—	—	10·0000	10·0000	62·0	53·5	29·309	
—	5 $\frac{52}{60}$	5 $\frac{52}{60}$ (1 day)	—	—	—	20·0117	20·0117	68·9	59·0	29·242	
1 $\frac{50}{60}$	3 $\frac{40}{60}$	5 $\frac{30}{60}$ (1 day)	—	—	5·4500	8·5500	14·0000	61·0	54·5	29·321	
1	4 $\frac{70}{60}$	5 $\frac{70}{60}$ (1 day)	—	—	1·5600	13·0200	14·5800	55·9	52·0	29·180	
Workshop experiments :											
21 $\frac{3}{60}$	5 $\frac{42}{60}$	7 $\frac{55}{60}$ (1 day)	31 $\frac{7}{60}$	2 $\frac{55}{60}$	8·4500	18·7500	27·2000	50·5	49·0	28·994	
21 $\frac{0}{60}$	25 $\frac{54}{60}$	28 $\frac{4}{60}$ (5 days)	211 $\frac{4}{60}$	4 $\frac{0}{60}$	8·1800	91·8500	100·0300	66·8	50·9	29·143 to 29·529	
6 $\frac{20}{60}$	22 $\frac{6}{60}$	28 $\frac{35}{60}$ (5 days)	20 $\frac{6}{60}$	2	24·2700	77·8500	102·1200	63·0	48·0	28·930 to 29·293	
—	24 $\frac{20}{60}$	24 $\frac{20}{60}$ (6 days)	24 $\frac{20}{60}$	—	—	67·2000	67·2000	71·9	58·1	29·249 to 29·716	

TABLE IX.

Showing the composition of a typical tinning metal and of the substance which collects as a pasty mass on the surface of the molten metal during tinning and from which vapours appear to emanate.

Nature of sample and how collected	Percentage composition				Ratio Tin Lead	
	Soluble in water		Insoluble in water		In sample	In original tinning metal
Substance which collects as a paste on surface of tinning metal. Taken from surface of molten metal at conclusion of Experiment IV	Zinc chloride	34·28 %	Tin oxide with trace of metallic		4·91	1
	Lead chloride	4·63	tin	47·12 %	1	1
	Iron chloride	0·98	Zinc oxide...	5·32		
			Lead oxide	4·43		
			Iron oxide	0·98		
			Chlorine (present as insoluble oxychloride)	1·67		
			Loss, etc....	0·59		
			Metallic tin	53·95	1·22	—
			Metallic lead	44·21	1	
Tinning metal. Taken from tinning bath at conclusion of Experi- ment VI			Metallic copper	1·13		
			Metallic iron	0·50		
			Metallic antimony	0·21		
			Metallic zinc	Nil		
			Metallic arsenic	Nil		

Distribution of deleterious substances in the atmosphere of a tinning workshop.

In order to form some conception of the manner in which air in different parts of the workshop is affected by the deleterious substances produced in tinning, samples of the dust, found deposited in all parts of the tinning workshop, were collected from selected points. In a good light it is possible to trace the general course of the fumes produced though this of course varies in different workshops. Usually, however, they appear to ascend to the roof immediately over their point of production without becoming disseminated to any great extent in the surrounding atmosphere. The workshop in which the experiments were made is very high and the blue vapours from the tinning baths and wiping stands appeared to rise directly upwards from their source of origin. In the upper part of the roof they appeared to mingle to some extent and then pass away through the large louvres provided. In the working portion of the atmosphere, *i.e.* for a height of 10 or 12 feet, vapour could only usually be observed in the immediate neighbourhood of the baths, etc. It was however impossible to say that dust (no doubt largely consisting of mineral matter produced in raking the fires) was more prevalent in one part of the atmosphere than another. Hence so far as ocular evidence can be trusted it appeared probable that all parts of the workshop are not equally dangerous. This point is emphasised by the result of Dr Legge's medical examination by which it was shown that while the men using a molten mixture of lead and tin were plainly affected by lead the worker using a pure tin bath in the same room was only slightly affected. The points from which the samples of dust were taken were selected with the object of testing the validity of this conclusion. The following table shows the point of collection and probable source of deleterious substances judged by ocular evidence of the course of the fumes. In taking the samples the whole of the dust from a selected area was removed, it was black in colour and appeared to contain considerable quantities of fibre similar to the tow used in wiping. A large proportion was however simply finely divided powder.

TABLE X.

Showing details of collection of dust samples.

Distinctive No. of Sample	Where collected	(a) Point from which collected	(b) Probable point of origin of deleterious substances
		(1)	(2)
10	¹ Tinning Work- shop A.	(a) Ledge of hood covering a common tinning bath, about 4 ft. 10 ins. above surface of molten metal, 5 ft. from wiping stand, and immediately in front of position of tinner during tinning.	(b) Fumes from tinning bath, fumes from articles when being removed from bath, and fumes and fibre from wiping stand.
11	Do.	(a) Ledge of window between common tinning and pure tin baths 8 ft. 6 ins. from floor, the vertical line from this point to the floor being 5 ft. 6 ins. from the open front of the pure tin bath and 4 ft. 4½ ins. from the side wall of the common tinning bath and about 10 ft. from the wiping stand.	(b) Principally fibre from the wiping stand, with some fumes from the pure tin bath.
12	Do.	(a) Window ledge 8 ft. 6 ins. from floor and immediately over the front of the pure tin bath, about 6 ft. above the surface of the metal.	(b) Fibre from the wiping stands and fumes from the pure tin bath.
13	Do.	(a) Middle of a beam across middle of the workshop 13 ft. 6 ins. from the floor.	(b) Fibre and fumes from wiping principally, together with some fumes from each of the baths.
14	Mounting Shop used in connec- tion with Work- shop A.	(a) Middle of beam across middle of mounting shop 13 ft. 6 ins. from floor. Broken panes in windows between mounting and tinning shops allow passage of fumes from tinning shop.	(b) Fumes and fibre from wiping stands and a quantity of fumes from all baths which tend to collect in upper part of tinning workshop.
15	Do.	(a) Ledge of fire-place behind mounter 5 ft. 6 ins. from floor.	(b) Dust from hammering of tinned kettles during mounting. This was the only sample collected which was free from fibre.
16	Tinning Work- shop B.	(a) Blades of fan exhausting air by means of ducts and hoods from a point over the wiping stands, and also from a pure tinning shop in which no zinc chloride solution is used, but only molten tallow, for fluxing the metal, and hydrochloric acid for cleaning.	(b) Fumes and fibre from wiping stands.
17	Do.	(a) Ledge of hood over common tinning bath, in workshop where a fan is used to exhaust air from a point above the wiping stands 5 ft. 6 ins. above surface of molten metal and immediately in front of position taken up by tinner during tinning, about 7 ft. from wiping stand.	(b) Fumes from bath, and fumes and fibre from wiping stand.
18	Do.	(a) Beams just above a hood connected with ducts and fan exhausting air from above a wiping stand 4 ft. 3 ins. above lower edge of hood and 13 ft. 9 ins. from floor.	(b) Fumes and fibre from wiping stand, and fumes from common tinning bath.
19	Do.	(a) Hole in wall 7 ft. above surface of molten metal and immediately over open front of a common tinning bath, direction of fan draught over wiping stand, however, tends to draw fumes away from this point.	(b) Fibre from wiping stand, and also fumes from tinning bath.

¹ Tinning workshop A is that in which the experiments were carried out.

In the following table the percentage composition of these samples is given :

TABLE XI.
Showing percentage composition of dust samples.

Dis- tinctive No. of sample	Where collected	Percentage composition											Foreign matters (fibre, dust, minerals &c.)
		Soluble in water						Insoluble in water					
		Chlorides of					Hydro- chloric acid	Oxides of					
		Lead	Tin	Zinc	Copper	Arsenic		Tin	Lead	Zinc	Copper	Arsenic	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
10	¹ Tinning Work- shop A.	9.26	0.14	5.83	0.32	Trace	1.39	17.12	5.42	0.31	0.41	Trace	59.80
11	Do.	0.11	Trace	0.79	0.08	Nil	0.11	9.56	5.83	0.28	0.30	Do.	82.94
12	Do.	0.08	0.06	1.75	0.08	Do.	0.15	5.77	3.41	0.45	0.28	Do.	88.07
13	Do.	0.71	Trace	2.56	0.25	Do.	0.82	8.72	6.17	0.28	0.12	Do.	80.37
14	Mounting Shop.	0.20	Do.	2.11	0.17	Do.	0.50	7.09	3.71	0.24	0.12	Do.	85.86
15	Do.	Nil	Do.	0.25	0.04	Do.	Nil	1.67	1.51	0.46	0.07	Do.	96.00
16	Tinning Work- shop B.	7.31	0.05	1.64	0.33	Do.	0.57	9.22	2.37	0.05	0.15	Do.	78.31
17	Do.	5.75	0.01	11.83	0.22	Do.	0.77	7.34	1.32	0.08	0.10	Do.	72.58
18	Do.	17.19	0.03	6.94	0.19	Do.	2.25	21.94	2.72	0.11	0.11	Do.	48.52
19	Do.	6.99	0.03	7.15	0.06	Do.	0.07	14.76	5.64	0.14	0.14	Do.	65.05

¹ Tinning workshop A was that in which the experiments were carried out.

In considering the composition of samples of dust it would appear at first sight that this should approximate to that of fumes aspirated from a point near that from which the dust was taken. This is however not necessarily correct since in the experiments the fumes were condensed as a whole and therefore the results show their true composition, while if left to themselves they probably condense fractionally, those compounds like lead chloride condensing more quickly than others. Moreover the experiments show the fumes to contain a large proportion of hydrochloric acid, a gas which would not be deposited to any great extent since the samples of dust were quite dry in the ordinary sense. It is certainly a fact however that the result of Experiment VIII shows that the composition of the fumes, etc. produced in wiping does approximate to that of the sample of dust (sample 10) taken from a point in close proximity to the wiping stand, provided that the extraneous matters, *e.g.* mineral dust and fibre, etc., are eliminated by calculation. In the following table the composition of the deleterious substances which, when deposited with foreign matters, form dust

samples, is shown. The results have been obtained by eliminating by calculation all foreign matters. The results of Experiment VIII similarly treated are also shown for purposes of comparison.

TABLE XII.

Showing percentage composition of deleterious matters as they probably exist in the air before deposition as dust.

Dis- nctive no. of mple.	Percentage composition of deleterious matters found in dust											Ratio $\frac{\text{Tin}}{\text{Lead}}$	
	Soluble in water						Insoluble in water					In sample	In original tinning mixture
	Chlorides of					Hydro- chloric acid	Oxides of						
	Lead	Tin	Zinc	Copper	Arsenic		Tin	Lead	Zinc	Copper	Arsenic		
xp.) 8	18.47	Trace	12.12	—	—	Nil	38.53	22.08	—	—	—	$\frac{0.89}{1}$	$\frac{1.22}{1}$
10	23.03	0.35	14.50	0.80	Trace	3.46	42.59	13.48	0.77	1.02	Trace	$\frac{1.14}{1}$	$\frac{1.22}{1}$
11	0.65	Trace	4.63	0.47	—	0.64	56.04	34.17	1.64	1.76	Do.	$\frac{1.37}{1}$	$\frac{1.22}{1}$ & $\frac{1}{0}$
12	0.67	0.50	14.67	0.67	—	0.42	48.37	28.58	3.77	2.35	Do.	$\frac{1.4}{1}$	$\frac{1.22}{1}$ & $\frac{1}{0}$
13	3.62	Trace	13.04	1.27	—	4.18	44.42	31.43	1.43	0.61	Do.	$\frac{1.1}{1}$	$\frac{1.22}{1}$ & $\frac{1}{0}$
14	1.41	Trace	14.92	1.20	—	3.54	50.14	26.24	1.70	0.85	Do.	$\frac{1.55}{1}$	$\frac{1.22}{1}$ & $\frac{1}{0}$
15	—	Trace	6.25	1.00	Trace	—	41.75	37.75	11.50	1.75	Do.	$\frac{0.94}{1}$	$\frac{1.22}{1}$
16	33.70	0.23	7.56	1.52	—	2.63	42.51	10.93	0.23	0.69	Do.	$\frac{0.95}{1}$	$\frac{1}{1}$?
17	20.97	0.03	43.14	0.80	—	2.81	26.77	4.82	0.29	0.37	Do.	$\frac{1.05}{1}$	$\frac{1}{1}$?
18	33.39	0.06	13.48	0.37	—	4.38	42.62	5.28	0.21	0.21	Do.	$\frac{1.12}{1}$	$\frac{1}{1}$?
19	19.98	0.09	20.44	0.17	—	0.20	42.20	16.12	0.40	0.40	Do.	$\frac{1.11}{1}$	$\frac{1}{1}$?

The results given in the two tables above are in many cases inconsistent. They however show that all parts of a tinning workshop are to a certain extent dangerous, and that points near the baths and wiping stands are exceedingly so (cf. samples 10, 17, 18 and 19). Samples 11 and 12 taken from a point remote from any source of lead fumes contain very little lead chloride though the influence of the pure tin bath is shown by the proportion of zinc chloride in the two samples and also by the increase in the ratio of tin to lead. Generally it appears from a study of the tables that the fumes do cling to the part of the workshop in which they are produced, this being most strikingly shown by the

decrease in the lead chloride found in those samples taken from a point remote from a source of lead fumes. The proportion of insoluble lead is fairly regular in all the samples taken from any particular workshop though it evidently varies under different conditions in different workshops. Thus in workshop B it is much lower than in workshop A while the proportion of lead chloride is generally higher. In the former, however, air was exhausted from the wiping stands and also from the pure tinning shop by means of a fan and main trunk with hoods over each stand. Probably a large quantity of the fibre produced in wiping is removed by this means but the construction and position of the exhaust was such that while not sufficiently strong to remove all the fumes produced during wiping it would yet have a considerable tendency to draw fumes out of the hood, covering the tinning bath, into the workshop. The effect is shown by the greatly increased proportion of lead chloride and decreased percentage of metallic lead though probably the tinning metal used which is richer in lead may have some effect in causing the difference. In general however the fan has a good effect since it was noticeable that much less dust was deposited than in any other workshop visited and the movement of air was sufficiently great to reduce very considerably the quantities of deleterious substances present in a given volume of air. The good effect is also shown by the low proportion of cases of lead poisoning occurring in this workshop. In workshop A the proportion of fibre was apparently about the same in all the samples and this is supported by the quantity of insoluble lead found in those samples (11, 12 and 13) not much affected by any source of fumes. The proportion of lead chloride found in these samples throws some light on the question of the quantity of this compound carried by fibre floating in the atmosphere. Assuming, as seems probable, that nearly all the insoluble lead found in the dust is carried by such fibre and putting down the whole of the lead chloride found in samples 11 and 12 as coming from this source it will be seen that the proportion of lead chloride in the atmosphere mechanically carried on fibre is very small as compared with that due to fumes or vapour near the tinning baths and wiping stands (cf. samples 11 and 12 with samples 8 and 10) and does not amount to more than $\frac{1}{40}$ or $\frac{1}{50}$ of the quantity of insoluble metallic lead carried in the same way and found in the dust samples or in Experiment VIII. The insidious nature of the fumes is shown by sample 14 taken from the mounting shop, they having apparently leaked from the tinning workshop through broken windows situated at a height of about 14 feet in the wall dividing the

two workshops. From a consideration of the composition of this sample, especially of the ratio of tin to lead, it is apparent that fractional deposition of the fumes must take place. Sample 15 shows there is a certain amount of danger from mounting, appreciable quantities of metallic tin and lead dust being produced by the hammering. A further point brought out by the examination of samples of dust is that compounds of copper, probably resulting from the chemical action of the flux and acid on the brazed part of the article being tinned, and also of arsenic, probably originating as impurity in the acid used, must exist in the air of tinning workshops.

THE DRINKING WATER OF STEAMSHIPS.

BY MARC ARMAND RUFFER AND J. GRAHAM WILLMORE.

*(From the Bacteriological Laboratories of the Sanitary,
Maritime and Quarantine Council of Egypt.)*

WE intend to give in this paper a short account of (1) the laws concerning the supply of drinking water to ships in some of the chief civilised countries of the world; (2) the results of bacteriological examination of the water on steamships; (3) the chief deficiencies in supply and storage which have come under our notice, and (4) the reforms which we consider urgently necessary.

(I) *Laws regulating the water supply on board steamships.*

*Great Britain*¹. Although in general no rules are laid down directly as to methods (1) of shipping, (2) of storing water in British ships, there are certain provisions which, by the threat of inspection or by penalties attaching to unsanitary methods, indirectly impose on Masters of ships the necessity of sanitary observances in these respects.

Under section 206, sub-section (2) of the Merchant Shipping Act of 1894 :

“in the case of ships going or trading from any port of the United Kingdom through the Suez Canal or round the Cape of Good Hope, or Cape Horn, the Inspecting Officer may at any time proceed on board any ship to ascertain whether the stores and water provided have been duly inspected, or if not, whether they are of a quality fit for the use of the crew of the ship, and if he finds the same not to have been inspected, and to be deficient in quality, the ship shall be detained until the defects are remedied according to his satisfaction.”

Under section 207, sub-section (4) of the same Act :

“If a seaman or apprentice is ill, and has, through the neglect of the master or owner of the ship, not been provided with proper provisions and water according to

¹ This information was obtained from M. C. Greig, H.M.'s Vice-Consul, Alexandria.

his agreement...then the owner or master, unless it can be proved that the illness has been produced by other cause, shall be liable to pay all expenses (not exceeding on the whole three months wages) properly and necessarily incurred by reason of the illness...."

Under section 198, sub-section (1)—(2) of the same Act :

"If three or more of the crew of a British ship consider that the provisions or water for the use of the crew are at any time of bad quality, they may complain thereof to any of the following officers, namely, an Officer in command of one of His Majesty's ships, a British Consular Officer, a Superintendent, or a Chief Officer of Customs, and the Officer may either examine the provisions or water complained of, or cause them to be examined."

"If the Officer or person making the examination finds that the provisions or water are of bad quality and unfit for use, or deficient in quantity, he shall signify it in writing to the master of the ship, and if the master of the ship does not thereupon provide other proper provisions or water in lieu of any so signified to be of bad quality and unfit for use, or does not procure the necessary quantity of any provisions or water so signified to be deficient in quantity, or uses any provisions or water so signified to be of bad quality and unfit for use, he shall for each offence be liable to a fine of not exceeding twenty pounds."

In the case of emigrant ships special regulations are enforced with regard (1) to the issue of water during the voyage, (2) to the mode of carrying water, (3) to the shipping of water at intermediate ports.

(I) As regards the *supply and issue of water during the voyage* the Merchant Shipping Act of 1894 makes the following provisions :

Section 295 (1), "There shall be placed on board every emigrant ship, for the steerage passengers, provisions and water of good and wholesome quality and in sweet and good condition, and in quantities sufficient to secure throughout the voyage the issues required by this part of the Act (*i.e.* 'three quarts daily to each statute adult, exclusive of the quantity necessary for cooking any article issued under this Schedule¹ in a cooked state')."

(2) "In addition to the allowance of pure water for each steerage passenger, water shall be shipped for cooking purposes sufficient to supply ten gallons for every day of the length of the voyage, for every one hundred statute adults on board."

(3) "There shall also be shipped for the use of the crew and all other persons on board an ample amount of wholesome provisions and pure water, not inferior in quality to the provisions and water provided for the steerage passengers."

(4) "All such water and provisions shall be provided and stowed away by and at the expense of the owner, charterer, or master of the ship."

(5) "If any emigrant ship obtains a clearance without being provided with the requisite quantities of water and provisions in accordance with this section, the owner, charterer, or master of that ship shall for each offence be liable to a fine not exceeding 300 pounds."

¹ If necessary read Schedule XII of the list in question.

(6) "Before an emigrant ship is cleared outwards, the emigrant officer at the port of clearance shall survey or cause to be surveyed by some competent person the provisions and water by this Act required to be placed on board for the steerage passengers, and shall satisfy himself that the same are of good and wholesome quality and in sweet and good condition, and in the quantities required by this Act."

(7) "If the emigration officer considers that any part of the provisions or water is not of a good and wholesome quality, or not in sweet and good condition, he may reject and mark the same or the vessels or packages in which it is contained, and direct the same to be forthwith landed and emptied."

(8) "If the same are not forthwith landed or emptied, or if after being landed or emptied the same or any part thereof are reshipped, the owner, charterer, or master of the ship shall for each offence be liable to a fine not exceeding one hundred pounds."

Section 298 (1), (2), (3), of the same Act provides as follows:

"The master of any emigrant ship shall, during the voyage, including the time of detention at any place before the termination thereof, issue to each steerage passenger, or where the steerage passengers are divided into messes, to the head man for the time being of each mess, on behalf of and for the use of all members thereof, an allowance of pure water and sweet and wholesome provisions of good quality, in accordance with the dietary scales in the 12 Schedule to this Act which shall have effect as if they were contained in this section."

"If any requirement of this section is not complied with in the case of any emigrant ship, the master of the ship shall for each offence be liable to a fine not exceeding fifty pounds."

(II) As regards the *mode of carrying water* section 296 (1), (2), of the same Act provides as follows:

"The water to be placed on board emigrant ships as hereinbefore provided shall be carried in tanks or casks approved by the emigration officer at the port of clearance, and the casks shall be sweet and tight, of sufficient strength, and if of wood, properly charred inside, and the staves shall be made of fir, pine, or soft wood, and each cask shall not be capable of containing more than 300 gallons."

"If any requirement of this section is not complied with in the case of any emigrant ship, the owner, charterer, or master of the ship or any of them, shall for each offence be liable to a fine not exceeding fifty pounds."

(III) Concerning the *shipping of water at intermediate ports* section 297 of the same Act provides as follows:

"If an emigrant ship is intended to call at the intermediate port during the voyage for the purpose of taking in water, and if an engagement to that effect is inserted in the master's bond hereinafter mentioned, it shall be sufficient to place on board at the port of clearance such supply of water as is required by this part of the Act for the voyage to the intermediate port, subject to the following conditions; that is to say (a) the emigration officer at the port of clearance shall approve in writing the arrangement, and the approval shall be carried among the ships' papers,

and shall be exhibited at the intermediate port and delivered on the arrival of the ship at the final port of discharge to the chief officer of Customs, or British Consular Officer, as the case may be: (b) if the length of either portion of the voyage, whether to the intermediate port, or from the intermediate port to the final port of discharge, is not determined under this part of this Act, the emigration officer at the port of clearance shall declare the same in writing as part of his said approval of the arrangement: (c) the ship shall have on board at the time of clearance such tanks and water casks of the description this part of the Act requires, as are sufficient for stowing the quantity of water required for the longest of the aforesaid portions of the voyage."

In most of the ports of the United Kingdom, *i.e.* Glasgow, Tyne-mouth, Southampton, London, Liverpool etc., there are no special regulations regarding the supply and storing of water on ships; according to Dr Hubert Williams, Medical Officer of the Port of London, a certain number of ships are supplied by water-barges. These water-boats are kept under careful observation, and their tanks are cleaned out and limewashed at regular intervals, and whenever, in the opinion of the Inspectors of the Port, it is deemed necessary.

*France*¹. There are no laws or regulations concerning the supply and storage of water on ships.

Inquiries made at Le Havre, Brest, Marseille, Boulogne, Dunkerque, and other French ports elicited the fact that the sanitary authorities did not concern themselves with the question.

Germany. We are informed by Dr Bumm, President of the Imperial Health Office in Berlin, that no imperial laws on the subject exist in Germany, but most harbour authorities provide stand pipes specially for ships. In Hamburg the port authorities give all captains a plan of the port, in which about twenty places, where good drinking water may be obtained gratis, are plainly marked.

On emigrant ships, iron tanks must be carried, the inner walls of which are coated with cement or some other suitable substance. In Hamburg, the water supply is under the direct control of the port medical officer, whose instructions contain a paragraph enjoining him: (1) to pay particular attention to the water supply of ships, (2) to see that water-boats carry water free from suspicion, and (3) that the tanks and pipes of such boats be always kept clean and in good order.

Similar instructions are given to the port medical officer of Bremen. The rules of Hamburg harbour forbid the use of Elbe water for drinking or cleaning the ship's eating, drinking and cooking utensils.

¹ Information obtained from M. Nettement, French Vice-Consul in Alexandria.

*Russia, Belgium, Holland, Portugal*¹. There are no laws whatever on this point in these countries. In Russia, however, certain companies have drawn up rules for the guidance of captains, but there are no imperial laws.

*Austria*². Passenger ships must carry water enough to supply each passenger with four to five litres of water per diem. The sanitary authorities pay special attention to this question, and from time to time examine samples of water both bacteriologically and chemically; if necessary, they order the water tanks to be emptied and cleaned. The medical authorities base their action on the fact that the law states that the captain is responsible for the good quality of the provisions of the ship; and drinking water is included under provisions.

*Sweden*³. The only law on this subject provides that every member of the crew and every emigrant must be provided daily with five litres of water for drinking and cooking purposes.

*Norway*⁴. According to Norwegian law, the drinking water should be stored in iron tanks, but this is compulsory in the tropics only. The tanks must be cemented or whitewashed inside and "perfectly clean."

The law states that iron-made tanks, which are used both as meat and water tanks, should be carefully cemented and should be cleaned with soda every year. Wooden tanks must be cleaned and whitewashed from time to time. There are also special recommendations regarding the place where water is taken, and with regard to boiling the water in harbours where there is yellow fever, cholera, dysentery, climate fever, beriberi or typhoid fever.

Bad water must be changed as soon as possible, and the tanks completely emptied and cleaned, before fresh water is taken in. This must be done also on ships going on a long voyage.

*Italy*⁵. In Italy, emigrant ships must carry enough fresh water to provide each person with five litres daily, unless there is a distilling machine on board. In any case enough water for three days must be stored always and in such a way that it cannot be contaminated. The reservoirs must be cleaned after each voyage and not refilled before the government medical officer or the "travelling commissioner" has inspected the tanks.

¹ Information obtained from M. d'Abazza, Russian Consul, M. Goor, Belgian Consul, Dr Demech, Dutch Delegate, Dr Mauri, Portuguese Delegate in Alexandria.

² Information obtained from Dr Osborn, Austrian Delegate in Alexandria.

³ Information obtained from Dr Kartulis, Swedish Delegate in Alexandria.

⁴ Information obtained from Dr Demitriadis, Norwegian Delegate in Alexandria.

⁵ Information obtained from Dr Torella, Italian Delegate in Alexandria.

Inquiries made at various Italian ports (*e.g.* Livorno, Brindisi, Ancona, Venice) show that there are no local regulations regarding the water supply of ships in the chief Italian ports.

Ports in the Far East. A similar state of things exists in most harbours of the Far East. In some places, however, precautions are taken for the regular cleaning of water cisterns. In Rangoon for instance the floating cisterns are "occasionally" examined by the Port Health Officer. In Calcutta the tanks of water-boats are kept under lock and key. At Perim the tanks on the water-boats are regularly cleaned and coated with a cement-wash every three months. In all the other places from which we have been able to get information the rule seems to be that no special precautions are taken to insure pure water reaching the ships.

A similar state of things existed in Egypt, up to a few months ago. We were unable to get much information with regard to America. The Port Medical Officer of New York, however, states that there are no laws concerning the water supply of ships in New York harbour.

(II) *Bacteriological examination of ship water.*

During the year 1906 the water of ships calling at Port Said, of a certain number of pilgrim ships calling at El-Tor, and of a few ships at Alexandria, was examined bacteriologically. In each case, notes were taken of the source of the samples, the methods of storing, of filling the tanks and the possibility of contamination on board.

Data bearing on the possibility of contamination could not always be obtained for various reasons; and in many ships the tanks were so situated that inspection during the voyage was not possible.

The water was plated out as soon as the sample, taken in a sterilized vessel, had been carried in an icebox to the laboratory. The plates were made on agar and gelatin in Petri dishes, and on Conradi-Drigalski's medium in some cases.

Three agar and gelatin plates were used as a rule, each containing respectively 0.1 c.c., 0.05 c.c. of the sample, and 0.1 c.c. of a 1 % dilution in sterilized water.

During August, gelatin was not used, and later on 50 %, 40 % and 20 % gelatins were prepared. The gelatin plates were usually left for 72 hours in the dark at the temperature of the room. The concentrated gelatins impeded the growth of some bacteria, so that our numbers are sometimes too low.

In all cases a flask containing peptone solution was inoculated at the time of taking the sample, and tested for indole at the end of five days at 37° C. No attention was paid to vibrios, as Dr Zirolia has already studied that subject.

No attempt was made to isolate particular species, except in a few instances. *Bacillus coli* is specially mentioned as having been present occasionally, and when this is the case, attention had always been drawn to its presence by the strong faecal odour, emanating from the peptone flask. This organism must have been present in many other samples, as when actually found no special methods had been necessary for its isolation.

It was easily recognised on Conradi-Drigalski plates and the diagnosis verified by the usual biochemical tests.

The table on pp. 514—519 shows the main results of our investigations.

Discussion of results.

The fact is to be borne in mind that many of the ships, from which samples of water were taken, were not cargo-boats or ocean tramps, but liners belonging to the best English, French and German companies. The water on such high-class passenger ships was as bad, if not worse, than on other ships.

The main results of this investigation therefore are that in only one ship was the water fit to drink, that not unfrequently there was evidence of faecal contamination, and that in some it was little better than diluted sewage.

In some cases, the water provided to the passengers had been filtered on board, but although the number of colonies was markedly diminished by this process, yet the water was by no means bacteriologically clean.

Source of contamination of the water.

The water supply of ships may be contaminated at three different times :

(1) *The water supply of the harbour may be contaminated.*

(2) *The water may be contaminated between the shore and the ship.*

This, owing to various reasons, is very often the case.

(a) *Deficiencies in hose.* When the water flows directly or is pumped through a hose from a standpipe to the ship, the hose may be the source of the evil. In harbours, the hose is often dragged along the ground,

through mud and dust, and screwed on without cleaning. Ruge (see Nocht, p. 223) has seen human excrements lying close to the standpipe. The hoses are frequently leaking and dirty water finds its way into them.

On one government ship we saw the men washing their heads and feet in the water which was flowing direct from the hose into the ship's water tank. Soapsuds, dirt, water and all went down together into the tank. Moreover, in many ships and harbours, there is no proper place for the storage of hoses.

(b) *Deficiencies in water cisterns.*

The danger is much greater when the water is taken on board ship by means of floating cisterns, owing to the dirty condition and faulty construction of the latter. In some instances, these are practically open tanks; the size and unprotected condition of the man-holes allowing direct contamination of water.

The wooden covers (for some boats are still provided with such) often fit badly and not uncommonly are absent altogether.

In one case, for instance, we saw a water lighter steaming slowly past a ship which was being washed down, after coaling. As the hose was raised, a stream of dirty water poured through one of the man-holes directly into the water tank.

It is not uncommon to find a thick layer of coal dust, and a plentiful crop of weeds on the surface of the water. The walls of the cistern are often covered with an adhering viscid material.

A few bacteriological examinations of the cisterns will show how greatly the water in them may be contaminated.

No. of water tank				Number of colonies in 1 c.c.
I. (Suez)	5580
II. (")	3720
III. (Port Said)	44470
IV. (")	13050
V. (")	14400
VI. (Alexandria)	500
VII. (")	800

The worst water was found in the water tanks supplying passenger ships trading with the East. That this water was unfit for drinking purposes was evidenced by the scum which was floating on its surface.

It can be shown that the water may become contaminated in the floating cisterns. In the following table, for instance, we have registered the number of colonies at the standpipe, and that of the same water after three days' storage on board water cisterns.

	Date . . . 29th March	30th March	April 1st
	Number of colonies		
Standpipe (<i>a</i>)	20	20	20
„ (<i>b</i>)	25	25	25
Water tank A (<i>a</i>)	280	380	740
„ „ (<i>b</i>)	170	290	800
„ „ B (<i>a</i>)	140	210	560
„ „ (<i>b</i>)	125	235	520
„ „ C (<i>a</i>)	240	350	640
„ „ (<i>b</i>)	255	345	620
„ „ D (<i>a</i>)	270	370	870
„ „ (<i>b</i>)	255	330	?

In another series of the experiments the number of colonies obtained was greater still, in one case rising from 5 per c.c. at the standpipe to 1920 per c.c. after three days.

(3) *The water may become contaminated on the ships themselves.*

(a) Deficiencies in supply pipe.

In many ships, particularly cargo-ships, the opening of the intake, or supply pipe, is flush with the deck and closed with a screw cap. In Eastern ports, these screw caps are usually removed when the ship is in port, in order to prevent their being stolen; and a wooden plug, usually wrapped round with a piece of dirty cloth or canvas, does duty instead. Often the supply pipe is simply left open.

We noticed for instance a ship which was taking in water and coal at the same time. The intake pipes of the above type were placed at the same level on the port and starboard sides of the ship. Both were open and the starboard tank was being filled from the lighter lying along the port side of the ship. The hose was leaking freely as is usually the case, and as the ship had a heavy list to port, the water from the hose, after washing the very dirty deck, was pouring into the unprotected port tank. In some ships the ventilating pipes, conveying air for the ship's suction pumps, are insufficiently protected, or omitted altogether, the air pressure being then obtained from the top of the tank.

(b) Deficient protection against outside contamination.

The tanks, though water tight as a rule, are not always protected against outside contamination. In some cases we have seen pilgrims and fourth class passengers camping over the tanks, their dirty bedding being spread over the man-hole. As this was not hermetically closed, faecal contamination must have taken place. In one ship indeed where this state of things was noted the water contained 243,400 colonies per cubic centimetre, including many colonies of *Bacillus coli*.

(c) Inefficient methods of cleaning.

The methods of cleaning ships' tanks are extremely crude. Generally the only method is that men enter the tank with bare and presumably dirty feet and possibly dirtier habits.

Whether all the stories told in this connection by naval officers are true or not we cannot say, but no one who has had experience with ships' crews will think them exaggerated.

(d) Faults in distilling machines.

These dangers are not nullified on ships, *e.g.* war ships, which carry distilling machines. Not to speak of the possibility of the water being contaminated after being distilled, it is a fact that many distilling machines are badly made and that the water is contaminated during the process of distillation. This fact is indeed well known to engineers. We have heard complaints, from crews of British war ships, regarding the foulness of the distilled water provided on board.

Many ships, although carrying a condenser, also take a certain quantity of water on shore and fill up with distilled water from day to day, when at sea. The resulting mixture may be appalling, although the passengers fondly believe that they are drinking distilled water.

(e) Faulty construction of tanks.

Most water tanks are so made that they cannot be emptied completely, and a residue amounting occasionally to several inches remains when the water is allowed to escape for cleaning purposes. This is due to the fact that there are no taps in the floors of the tanks. As a result, water, however clear and pure at the time of introduction, is immediately fouled by the residue of the old water left in the tank.

The distribution of water to passengers on board ships also leaves much to be desired. The water is generally unfiltered and when there is a filter on board it is often a useless, if not dangerous, charcoal filter. The bottles of water are frequently far from clean and the water is often allowed to stand in bottles from one voyage to the other, during the ships' stay in port. We have seen more than once larvae of mosquitoes and insects in the washing and drinking water on ocean-going steamers. Lastly, on some ships carrying emigrants and pilgrims from Eastern ports, deck passengers can only drink by sucking water up through tubes in direct communication with water tanks. The danger of this proceeding need not be emphasised.

No.	From what Port	Source of sample taken	Method of filling tanks	Method of stowing water on board	Possibility of contamination on board
1	Bombay	Aden	Hose from lighter	In iron tanks cemented	Improbable
2	Colombo	Colombo & Port Said	Hose from lighter	Iron tanks cemented	Probable
3	Calcutta	Calcutta	Hose from quay	Iron tanks cemented	Probable
4	Manilla via Bombay	Suez (mixed)	Hose from lighter	Iron tanks cemented	
5	Colombo	Colombo	Hose from lighter	Iron tanks cemented	Probable, opening of intake pipe flush with deck
6	Nagasaki Singapore Colombo	Colombo	Hose from lighter	Iron tanks and double bottom	Improbable
7	Singapore	Singapore	Hose from quay	Iron tanks cemented	Probable, unprotected air pipe communicating with tanks, flush with deck
8	Surabajia	Condensed		Iron tanks cemented	Probable, opening of intake pipe flush with deck
9	Yokohama	Colombo & Penang	Hose from lighter	Iron tanks cemented	Possible
10	Karachi	Karachi	Hose and hydrant from quay	Iron tanks cemented	
11	Bombay	Bombay	Hose and hydrant from quay		
12	Yokohama Burma Colombo	Mixed	Lighter containing casks and small iron tanks in Colombo	Iron tanks cemented	Intake pipe opening on wooden bulkhead about 2½ inches above deck
13	Karachi	Karachi	Hose and hydrant from quay	Iron tanks cemented	Probable, opening of intake pipe flush with deck
14	Calcutta	Calcutta & Colombo	Hose from lighter	Iron tanks cement washed	
15					
16	Port Said	Port Said Water-lighter			Large man-holes on deck communicating directly with interior of tanks, supplied with ill-fitting wooden covers which appear to be habitually unused. Scum on surface of water
17	Odessa	Clazomene	Hose and lighter	Iron tanks cemented	Highly probable, intake pipe through port-hole in steerage and through large iron man-hole in floor of deck below steerage, which was full of Russian pilgrims, and in a very dirty condition
18	Sinope	Distilled			Improbable
19	Hamburg	Hamburg	(Water steamer)	Iron tanks cemented	Improbable. Intake pipes on outside opening well protected and communicating directly with tanks
20	Bremen	Bremen		Iron tanks cemented	Improbable. Intake pipe as in note 19
21	Hamburg	Hamburg	Company's lighter	Iron tanks cemented	Improbable, vide 19 for one tank on top of tank, abaft engine room raised some 4 feet from floor of deck and with proper iron covers
22				Vide 35	Vide 35
23	Birkenhead	Birkenhead	Hose and hydrant from quay	Iron tanks cemented	
24		Condensed		cleaned out 3 weeks ago	

Number of colonies		Reaction on Conradi Dri- galski plates		Gas	Indole	Month	Remarks
Agar	Gelatin						
3 unfilt. 0 filt.		Acid	Alkaline		3	Aug.	Contains a few vibronic forms and a very vigorous growth of <i>B. fluorescens putidus</i> . Impossible to count colonies in gelatin owing to rapid liquefaction by <i>B. fluorescens</i> and heat combined.
0056					3	Aug.	Contains <i>B. fluorescens</i> . Temp. of laboratory too high to admit of accurate counting of gelatin colonies.
132 200				+	2 3	Aug. Aug.	Contains approximately 1 <i>B. coli communis</i> to the drop. Had previously taken water in Yokohama and Bombay.
130		+			1	Sept.	
0 filt. unfilt.					0 3	Sept.	Troopship.
340	32430				3	Sept.	Numerous vibrios, no cholera-red reaction.
004	12465				0	Sept.	
900	16700				$\frac{1}{2}$	Sept.	Water supply renewed in Shanghai, Penang, and Colombo.
829	11875				2	Sept.	
990	15435				0	Sept.	
170	65430				2	Sept.	Some Yokohama water left in tanks, condensed from Burma to Colombo. Sample contains Port Said water mixed with above.
196	8050				$\frac{1}{2}$	Sept.	
480	14086				0	Oct.	
440 470	2090				2 3	Oct. Oct.	Numerous rose-red chromogenetic bacteria.
0 filt.	20480 filt. 660 liquefy- ing colonies	+		+	3	Dec.	
390	110	-	+	-	0	Dec.	Had taken on Russian pilgrims from the "Gregory Merck" and had had cholera on board since leaving Sinope. Vibrios isolated from one case. Culture taken at time of our visit. Water distilled in apparatus said to be of English make, no vibrios in peptone solution from sample of water.
320	630	trace	-	-	0	Dec.	Supply taken on in Hamburg from Company's own well by Company's water steamer.
400 200	220	-	+	-	1	Dec.	
0 filt. 0 filt.	21400 filt. 40940 filt.	+	-	+	2	Dec.	
		+	++	+	3	Dec.	
315	16060 liquefying 420	+	+	0	0	Dec.	The growth appeared only after 48 hours incubation, examination also showed majority of bacteria present to be sporogenic bacilli.

No.	From what Port	Source of sample taken	Method of filling tanks	Method of stowing water on board	Possibility of contamination on board
25		Condensed, but from tanks which had contained Malta water			
26		Condensed			
27	Jeddah	Suez	Hose and lighter	Iron tank cemented of 135 tons capacity divided into 4 sections, each cut off from the others	Possible, owing to improper position of intake pipe
28	Jeddah	Distilled		Iron tanks cemented, not inspected	
29	Jeddah	Port Said	Hose and lighter	Iron tanks cemented	Probable, large man-hole leading directly to tanks in the main deck in the midst of the pilgrims' quarters. Opening closed by wooden cover slightly raised above level of deck
30	Jeddah	Constantinople	Hose and lighter	Iron tanks cemented	Probable, the opening of the intake pipe into one tank raised on board about 2 inches above the deck, other pipes open flush with deck
31	Jeddah	Odessa & Sevastopol		Iron tanks cemented	Probable, opening of intake pipe flush with deck
32	Port Said	Port Said			Vide no. 16
33	Port Said	Port Said			Vide no. 16
34	Bombay	Bombay	Hose and hydrant from quay	Iron tanks cemented	Impossible, opening of intake pipe raised 1 foot above level of deck
35	Yokohama	Mixed	Lighter	Iron tanks cemented	Highly probable, intake pipes flush with deck, tanks aft in ship's double bottom, closed with ill-fitting lids, accessible to contamination by carpenter or carpenter's mate (Chinese)
36	Bombay	Mixed	See Remarks	Iron tanks cemented	Probable, intake pipes flush with deck
37	Brindisi	Brindisi well	Hose and lighter	Iron tanks cemented	
38					
39	Java	Sumatra	See Remarks	Iron tanks cemented	Probable, opening of intake pipe flush with deck, brass caps to stowage replaced while in port with ill-fitting wooden plugs wrapped in oily clothes, these not removed while deck was washed down
40	Colombo	Colombo	Lighter and hose	Iron tanks cemented	Probable, opening of intake pipe flush with deck
41	Colombo	Mixed	See Remarks	Iron tank cemented	Possible, opening of intake pipe flush with deck
42	Kobe	Calcutta	Kobe, closed lighters Calcutta, hose and hydrant from quay	Iron tanks cemented	Impossible

Number of colonies		Reaction on Conradi Dri- galski plates		Gas	Indole	Month	Remarks
Agar	Gelatin	Acid	Alkali				
12430	25935 liquefying colonies 790	0	+	0	0	Dec.	24, 25 and 26 are warships.
15860	10800 no liquefying colonies in gelatin						Sample shows heavy precipitate of AgCl with AgNO ₃ showing presence of NaCl: there had been complaints on board as to taste and smell of this water which was distilled from Port Said harbour water (Naval basin).
2400	13200	-	+	-	-	Feb. '08	Pilgrim ship from Jeddah. Had taken no water since leaving Suez on the 22nd Dec. '07. No vibrios present. Ship very clean.
3800	26300	+	-	-	-	Feb. '08	Pilgrim ship from Jeddah. Had taken water in Constantinople. No vibrios present. Ship moderately clean.
9100	37400	+	-	+	3	Feb. '08	Ship conveying pilgrims from Jeddah. Was in a very dirty condition. This sample contained no vibrios, but large numbers of <i>B. pyocyaneus</i> and <i>B. coli communis</i> . The latter species constituted the majority of organisms present.
							Pilgrim ship, very dirty. Sample contained no vibrios, but a very large number of <i>B. coli communis</i> and <i>B. pyocyaneus</i> . The latter producing a large amount of deep green pigment.
							Pilgrim ship, very clean. No vibrios.
13050	16130				3	Oct.	
14400	5195				3	Oct.	
7870	20048				3	Oct.	
18494	14050				3	Oct.	Ship had taken water in Yokohama, Kobe, Hong-Kong, Saigon, Singapore, Colombo and Suez, the sample taken consisted of probably the remains of all these waters and distilled water which had been used to replenish the supply. There was a visible scum on the surface of the water in the tanks. Vigorous growth of <i>B. pyocyaneus</i> present.
7420	5595				2	Oct.	<i>Pyofluorescens</i> present. Sample consisted of water from Bombay, Chittagong, and Colombo, taken in from hose and hydrant at Bombay and from covered lighters at Chittagong and Colombo.
144500	50125				3	Oct.	Next to no liquefying colonies on gelatin.
36190	7845				2	Oct.	This would denote an excess of anaerobic bacteria.
glucose agar 259875 11570	925 liquefying colonies. Total number not estimated				3	Sept.	Ship had taken water in Surabajia (Java) and Penang, from closed lighter in Surabajia and from hose and hydrant in Penang, vibrios present in water. Peptone solution did not give cholera-red reaction. There had been a certain amount of diarrhoea among the crew during the voyage, two men were still suffering from it. On glucose agar 209250 colonies per c.c.: i.e. excess of anaerobic bacteria.
7595	2530				0	Sept.	
glucose agar 2760							
8655 filt.	773 filt.				3	Sept.	<i>Pyocyaneus</i> very deep green pigment present. Ship had taken water in Hamburg, Antwerp, Cape Town, Sydney, Freemantle and Colombo. Sample taken probably represented remains of all these waters.
glucose agar 1600 filt.							Water had been taken in from lighters in Hamburg, Freemantle and Colombo, in other ports by hose
2313 unfilt.							[and hydrant.
575	983				0	Sept.	Contains <i>B. subtilis</i> .
glucose agar 550							

No.	From what Port	Source of sample taken	Method of filling tanks	Method of stowing water on board	Possibility of contamination on board
43	Singapore	Singapore	Hose from quay	Iron tanks with a special patent cement	Improbable
44	Singapore	Distilled		Iron tanks with a special patent cement	Improbable
45	Karachi	Karachi	Hose and hydrant from quay	Iron tanks cemented	Improbable
46	Batavia	Singapore	Hose from quay	Iron tanks cemented	Probable, opening of intake pipe flush with deck
47	Calcutta	Calcutta & Colombo	Lighter and hose	Iron tanks cemented	Probable, opening of intake pipe flush with deck and brass caps replaced while in port with ill-fitting wooden plugs
48	Zanzibar	Zanzibar & Aden	Hose and lighter	Iron tanks cemented	Very improbable, intake pipe fitted to side of ship
49	Bombay	Bombay & Karachi	Hose and hydrant from quay	Iron tanks on deck cemented	Possible, intake pipe flush with bridge deck
50	Calcutta	Calcutta	Lighter and hose	Iron tanks cemented	Possible, opening of intake pipe flush with deck
51	Bombay	Bombay	Hose and hydrant from quay	Iron tanks cemented	Opening of intake pipe raised 2 ins. above the deck
52	Surabajia	Surabajia	Lighter and hose	Iron tanks cemented	
53	Calcutta	Calcutta	Hydrant on quay	Iron tanks cemented	
54	Sydney	Aden	Giddipore Docks	Iron tanks cemented	
55	Surabajia	Aden	Lighter and hose	Iron tanks cemented	Improbable, opening of intake pipe in engine room
56	Karachi	Karachi	Iron tanks sealed in lighter	Iron tanks cemented	Improbable
57	Bombay	Bombay	Hydrant and hose from quay	Iron tanks cemented	
58	Brisbane		Hose and hydrant from quay	Iron tanks cemented	
59	Marseilles	Marseilles		Iron tanks cemented	Improbable, intake pipes protected
60	Yambo	Suez	Hose and lighter	Iron tanks sealed and cemented	Intake pipes flush with deck
61	Yambo	Suez	Hose and lighter	Iron tanks cemented	Tanks under main deck, forward and aft. Large hatches in main deck securely fastened and caulked. Underneath a properly fitting iron man-hole, closed rubber washer and dog clamp. Ventilating pipe. No much likelihood of contamination through above. Intake pipes flush with deck
62	Yambo	Suez	Hose and lighter	Iron tanks cemented	Main storage tanks down in fore peak, under main deck. Tanks are simply protected by ill-fitting wooden hatches, not caulked or rendered water tight in any way: on top of this Arab firemen etc. have their quarters. Under the wooden hatch in a direct line are the usual iron man-holes, which are not properly closed. The ventilation is effected by a hole in the covers, directly under the rotten hatches mentioned opening directly into the tanks and not protected from contamination in any way. There is no ventilating pipe upon deck. The tanks, of which there were several, are connected together by pipes awash in bilge water, so that contamination in one tank is liable to spread to all

Number of colonies		Reaction on Conradi Dri- galski plates		Gas	Indole	Month	Remarks
Agar	Gelatin						
8635 cose agar	8520	Acid	Alkali		$\frac{1}{2}$	Sept.	Excess of anaerobic bacteria.
16140 40	12				0	Sept.	
9780 cose agar	8273				1	Sept.	Captain landed at Port Said and died of enteric fever.
7350 115950 cose agar					3	Sept.	Had taken water Batavia, Samarang and Singapore. Contains numerous liquefying colonies, which liquefied the gelatin plates too rapidly to permit accurate counting.
61200							
6520 cose agar	11666				2	Sept.	
10450							
580 cose agar	7220				2	Sept.	In gelatin, much liquefaction and numerous chromogenetic colonies.
990							
8270	6640	+	+	+	2	Oct.	<i>B. fluorescens</i> prominent but little liquefaction of gelatin.
7600	37345	-	+		1	Oct.	
28380	12216	+	-	+	2	Oct.	
00460	181440	+	-	+	4	Oct.	
76416	38610	trace	+	0	0	Oct.	
56240	179050	+	-	+	3	Oct.	
73444	210688	-	+	0	0	Oct.	
5200	12000	trace	+	0	trace	Oct.	
11962	9870	+	+	+	2	Oct.	
66950	43560	+	+	+	2	Oct.	
160 unfilt.	93100 unfilt.	+	0	+	2	Dec.	Indole present in both. On evaporation samples left a brown deposit which charred on ignition. <i>B. coli communis</i> present.
990 filt.	19053 filt.						
97400	124600	+	+	-	1	Mar.'08	Pilgrim ship, no vibrios.
67600	117300	+	-	+	3	Mar.'08	<i>B. coli communis</i> present. Pilgrim ship. No vibrios.
176700	243400	+	+	+	5	Mar.'08	<i>B. pyocyaneus</i> and <i>B. coli communis</i> present, the latter in enormous numbers. The ship was very crowded and in an extremely filthy condition. No vibrios.

The measures which in our opinion might remedy this dangerous state of things are as follows :

(1) The source of the water must be above suspicion. Ships therefore should be allowed to take water only in places licensed by the medical officer of the port.

(2) The hoses, pumps, water-boats, etc., should be the property of the port sanitary authorities, who would then be responsible for the proper storing and cleaning of such apparatus. Should this be impossible, no apparatus or water tank should be used, until it has been examined and declared satisfactory by the said authority. All the apparatus should be examined and tested once a month.

(3) Intake pipes flush with the deck should be absolutely prohibited. They should be replaced by upright iron pipes, three feet high, of standard gauge, having their free ends bent downwards and fitted with screw cap chained to the pipe. When in use the cap should be removed and the hose screwed on. Some simple device would indicate when the tank was full.

(4) All water tanks on board ships should be made of iron, and coated inside with cement.

(5) All water tanks should be placed so as to allow inspection at any time during the voyage.

(6) All water tanks should be so closed as to avoid all chances of contamination during the voyage.

(7) All water tanks should be fitted with ventilating pipes which should be carried up to a sufficient height to prevent contamination.

(8) One or more taps should be placed through the floor of tanks to insure the latter being completely emptied at regular intervals. The tanks should be constructed with "round corners"; the floor of the tanks should be sloping towards the taps used for emptying the tanks.

(9) The tanks should be completely emptied and disinfected after each voyage. The methods used at present for this purpose are extremely unsatisfactory. Some form of gaseous disinfection would probably give the best results.

(10) In order to insure these rules being carried out, an international agreement is necessary.

SOME EXPERIMENTS ON IMMUNITY AGAINST VACCINIA IN ANIMALS.

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HITHERTO all efforts to set up immunity against vaccinia in animals have been restricted to the vaccination of animals in the customary manner, or to the injection of vaccine lymph into them.

In the present series of experiments an attempt was made to derive from calf vaccine an unorganised body capable of causing specific immunity against vaccinia in animals.

The method of production of such an immunisator has been :—

1. Collection of vaccine pulp from a calf.
2. Trituration of this pulp with normal saline solution.
3. Heating this mixture at 60° C. for 1 hour, in some instances as noted below.
4. Storage of this mixture.
5. Filtration of this mixture.

In the first experiment guinea-pigs were used. Into these animals filtrates of autolysed lymph were injected subcutaneously as detailed in the following Table I. Six varieties of filtrates were prepared from the pulp of one calf as follows:—

(a) *Unheated portion of mixture.*

1. Passed through a Berkefeld filter.
2. " " Chamberland filter.
3. " " Martin's gelatin filter.

(b) *Heated portion of mixture.*

4. Passed through a Berkefeld filter.
5. " " Chamberland filter.
6. " " Martin's gelatin filter.

Each of these six filtrates was injected subcutaneously at intervals into a series of eight pigs. Subsequently these pigs were vaccinated in the usual manner with one strain of lymph, as were eight control pigs which had received no injection; and the result of such vaccination was noted daily for a week.

The following Table I shows the results obtained.

TABLE I. (8 guinea-pigs in each cage.)

+ = Specific vesiculation. ? = Specific reaction not amounting to vesiculation.
- = No specific reaction.

No. of cage	Material injected	Injections			Date of vaccination	Result of vaccination
		April 4	April 22	April 29		
23	Nil (control pigs)	nil	nil	nil	May 6	8+
25	Berkefeld V filtrate of heated autolysed lymph	1 c.c.	1 c.c.	4 c.c.	„	7- 1?
26	Chamberland filtrate of heated autolysed lymph	„	„	„	„	5- 3?
27	Martin's gelatin filtrate of heated autolysed lymph	„	„	„	„	6+ 2-
28	Berkefeld V filtrate of unheated autolysed lymph	„	„	„	„	6- 2+
12	Chamberland filtrate of unheated autolysed lymph	„	„	„	„	6- 2+
22	Martin's gelatin filtrate of unheated autolysed lymph	„	„	„	„	6+ 2--

The results obtained with the control pigs differed markedly from those which had received subcutaneous injections of Berkefeld and Chamberland filtrates in that while the controls all developed good vesicles, the others consistently failed to develop vesicles, and only occasionally showed some modified specific reaction. Again, the pigs injected with Martin's gelatin filtrates as a rule showed vesiculation though possibly some immunisation had been set up by these filtrates. The immunisation set up by heated autolysed filtrates seemed on the whole to be better than that set up by unheated ones.

Experiment 2 was made on the same lines as the foregoing with the results detailed in Table II.

The results obtained by this second experiment bring out, if anything, more clearly, the points shown by experiment 1.

Experiment 3. A calf was injected subcutaneously with a Berkefeld filtrate of a heated autolysed vaccine. On July 10th, 10 c.c. were

injected; on July 23rd, 10 c.c.; and on July 30th, 15 c.c. On August 7th the calf was vaccinated. No vesiculation resulted. Good vesiculation occurred on a control calf vaccinated with the same lymph.

TABLE II. (8 guinea-pigs in each cage.)

+ = Specific vesiculation. ? = Specific reaction not amounting to vesiculation.
- = No specific reaction.

No. of cage	Material injected	Injections			Date of vaccination	Result of vaccination
		May 13	June 4	June 11		
23	Nil (control pigs)	nil	nil	nil	July 8	8 +
25	Berkefeld V filtrate of heated autolysed lymph	2 c.c.	2 c.c.	2 c.c.	„	7 - 1 ?
26	Chamberland filtrate of heated autolysed lymph	„	„	„	„	6 - 2 ?
27	Martin's gelatin filtrate of heated autolysed lymph	„	„	„	„	5 + 1 ? 2 -
28	Berkefeld V filtrate of unheated autolysed lymph	„	„	„	„	7 - 1 ?
12	Chamberland filtrate of unheated autolysed lymph	„	„	„	„	7 - 1 +
22	Martin's gelatin filtrate of unheated autolysed lymph	„	„	„	„	4 + 2 ? 2 -

Experiment 4. A monkey was injected with a Berkefeld filtrate of heated autolysed vaccine. Subcutaneous injections were given:— November 25th, 10 c.c.; November 27th, 10 c.c.; November 30th, 10 c.c. On December 6th the monkey was vaccinated on a shaved area over the scapulae in linear incisions. On December 13th, the eighth day after vaccination when vesiculation should be at its height, two or three small round abortive vesicles, each about the size of a split pea, were visible at intervals along the lines of incision. On December 10th these vesicles had dried up. Proliferation of tissue forming a heaped up crust along each incision followed, but no further vesiculation.

Experiment 5. A monkey was injected subcutaneously with a Berkefeld filtrate of autolysed heated vaccine. On Jan. 20th, 15 c.c. were injected; on Jan. 23rd, 30 c.c.; on Jan. 28th, 15 c.c.; and on Jan. 31st, 30 c.c. On Feb. 13th the monkey was vaccinated. On Feb. 20th a few small abortive vesicles were visible as in experiment 4, along the lines of incision, but these were even less marked than those in experiment 4.

The evidence afforded by this limited number of experiments points to the facts:—

1. That it is possible to produce from calf vaccine a specific unorganised immunisator.

2. That this immunisator is of such a nature as to be capable of filtration through a Berkefeld V or a Chamberland filter, and possibly in some small degree through a Martin's gelatin filter.

The fact that such an immunisator can be obtained, naturally suggests that it might be capable of setting up immunity against small-pox. It has not yet been found possible, however, owing to the scarcity of small-pox material, to carry out any work on these lines. Experiments will be made at the first opportunity. It is evident that if an immunisator against small-pox could be prepared from calf-vaccine its use would lead, in all probability, to an appreciable gain in time in dealing with small-pox contacts. Usually, of course, such contacts are vaccinated, and when the vaccination is successful an immunisator is manufactured in the vaccinated area of the patient, which immunisator is absorbed and gives rise to protection. If, however, the immunisator could be given directly to the contact, the time necessary for him to manufacture his own immunisator would be saved, and such gain in time might be important. Furthermore, the injection of the immunisator could, if desired, be used in addition to, and not instead of, vaccination in these cases. The immunisator might also possibly be injected advantageously during the course of small-pox. Until investigation on these lines is possible, other points in connection with the behaviour of the immunisator are being worked at.

THE INFLUENCE OF TEMPERATURE, AND SOME OTHER PHYSICAL CONDITIONS, ON CALF VACCINE.

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1. TEMPERATURE.

THE effect of temperature on calf vaccine is a matter both of scientific interest and of economic importance in preventive medicine. The latter point is of especial significance in hot climates, where rapid deterioration of the potency of lymph is not uncommon; whilst, irrespective of latitude, knowledge of the optimum temperature at which vaccine lymph can be stored is much to be desired.

Work on these lines with vaccine has been done by Blaxall (1902), Blaxall and Fremlin (1906), and Carini (1906); and with vaccine and various organisms by Macfadyen and Rowland (1900).

It seemed desirable, however, to extend the scheme of work as regards calf vaccine adopted by these observers, and to some extent to amend them. It seemed worth while, for instance, instead of exposing one vaccine in the form of dry powder at 60° C., and another as glycerinated suspension at 10° C., and so on, to take for each experiment vaccine collected from one calf; to prepare this in a number of ways, and to ascertain the behaviour of these preparations under different temperature conditions.

In the present experiments vaccine from one calf has been used in the following forms:—

(a) *Crude exuded lymph.* This is the exudate expressed from vesicles 120 hours after vaccination of the calf, stored in sealed capillary tubes. This lymph generally coagulates soon after collection, the coagulum adhering to the sides of the tube. The fluid portion, separated from the coagulum, sealed up in capillary tubes was used for experiment.

TABLE I.

		59-60° C.										36-37° C.					10-15° C.					3-4° C.					-3--4° C.			
		Minutes					Days					Weeks					Weeks					Weeks								
		1	5	10	15	20	30	60	1	5	8	12	20	4	8	12	25	52	4	8	12	25	52	4	8	12	25	52	25	52
1	No. of Experiment																													
	Preparation of vaccine																													
	Exudate	+	+	+	+	?	-	-	+	+	+	+	+	+	+	+	+	?	+	+	+	+	?	+	+	+	+	+	+	+
	Glycerinated	+	+	+	?	-	-	-	+	+	+	+	?	+	+	+	+	?	-	+	+	+	?	+	+	+	+	+	+	+
	Chloroformed	+	+	+	?	-	-	-	+	+	+	?	?	+	+	+	+	-	-	+	+	+	?	+	+	+	+	+	+	+
2	Dry powdered	+	+	+	+	+	?	?	+	+	+	+	+	+	+	+	+	-	-	+	+	+	?	+	+	+	+	+	+	+
	Lanolinated	+	+	+	+	+	+	?	+	+	+	+	+	+	+	+	+	-	-	+	+	+	?	+	+	+	+	+	+	+
	Exudate	+	+	+	?	-	-	-	+	+	+	+	?	+	+	+	+	?	?	+	+	+	+	+	+	+	+	+	+	+
	Glycerinated	+	+	-	-	-	-	-	+	+	+	-	-	+	+	+	+	-	-	+	+	+	?	+	+	+	+	+	+	+
	Chloroformed	+	+	?	-	-	-	-	+	+	+	?	-	+	+	+	+	?	-	+	+	+	?	+	+	+	+	+	+	+
3	Dry powdered	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	?	-	+	+	+	?	+	+	+	+	+	+	+	+
	Lanolinated	+	+	+	+	+	+	?	+	+	+	+	+	+	+	+	+	?	?	+	+	?	+	+	+	+	+	+	+	+
	Exudate	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	?	?	+	+	+	+	+	+	+	+	+	+	+
	Glycerinated	+	+	-	-	-	-	-	+	+	+	-	-	+	+	+	+	-	-	+	+	+	?	+	+	+	+	+	+	+
	Chloroformed	+	+	?	-	-	-	-	+	+	+	-	-	+	+	+	+	+	-	-	+	+	?	+	+	+	+	+	+	+
4	Dry powdered	+	+	+	+	+	+	?	+	+	+	+	+	+	+	+	+	?	?	+	+	?	+	+	+	+	+	+	+	+
	Lanolinated	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	?	?	+	+	?	+	+	+	+	+	+	+	+
	Exudate	+	+	+	-	-	-	-	+	+	+	+	?	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+
	Glycerinated	+	+	-	-	-	-	-	+	+	+	-	-	+	+	+	+	-	-	+	+	?	+	+	+	+	+	+	+	+
	Chloroformed	+	+	?	-	-	-	-	+	+	+	-	-	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
5	Dry powdered	+	+	+	+	?	?	-	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Lanolinated	+	+	+	+	?	-	-	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Exudate	+	+	+	+	-	-	-	+	+	+	?	?	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Glycerinated	+	+	-	-	-	-	-	+	+	+	?	-	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Chloroformed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Dry powdered	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Lanolinated	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Exudate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Glycerinated	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Chloroformed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Dry powdered	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Lanolinated	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Exudate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Glycerinated	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Chloroformed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Dry powdered	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Lanolinated	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Exudate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Glycerinated	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Chloroformed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Dry powdered	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Lanolinated	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Exudate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Glycerinated	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Chloroformed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Dry powdered	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Lanolinated	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Exudate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Glycerinated	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Chloroformed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Dry powdered	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Lanolinated	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Exudate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Glycerinated	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Chloroformed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Dry powdered	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Lanolinated	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Exudate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Glycerinated	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Chloroformed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Dry powdered	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Lanolinated	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Exudate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Glycerinated	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Chloroformed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Dry powdered	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Lanolinated	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Exudate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Glycerinated	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Chloroformed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Dry powdered	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Lanolinated	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Exudate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Glycerinated	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Chloroformed	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Dry powdered	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	?	?	+	+	+	+	+	+	+
	Lanolinated	+	+	+	+	+	+	+	+	+	+	+	+																	

+ = Vesiculation. ? = Specific reaction not amounting to vesiculation. - = Absence of specific reaction.

(b) *Glycerinated calf vaccine*. This is a suspension of one part by weight of finely triturated vaccine pulp in four parts by weight of a 50% glycerine and water solution.

(c) *Chloroformed calf vaccine*. This is a suspension of one part by weight of finely triturated vaccine pulp in two parts by weight of distilled water, subjected to the action of chloroform vapour and air for from 10 to 15 minutes at 20° C. with subsequent removal of all chloroform and the addition of two parts by weight of glycerine.

(d) *Desiccated calf vaccine*. This is vaccine pulp reduced to dry powder.

(e) *Lanolinated calf vaccine*. This is a mixture of one part by weight of calf vaccine pulp with four parts by weight of lanolin.

All these were placed in sealed capillary glass tubes except (e) which, on account of its consistency, had to be placed in a wider-mouthed vessel of about 0.25 c.c. capacity which was hermetically sealed.

These tubes were placed at 59°—60° C., 36°—37° C., 10°—15° C., 3°—4° C. and - 4° C. for varying intervals of time. The contents of the tubes were inoculated on a calf 24 hours after the exposures had finished, and the results of such inoculations were noted 120 hours later.

The results obtained in these experiments are shown in Table I.

Table II is obtained by an analysis of the results in Table I, and gives the average result of the five experiments, and shows that whereas glycerinated lymph, whether treated previously with chloroform or not, retained its infectivity in a very similar manner under the various conditions of temperature; the other three varieties of material behaved somewhat differently.

TABLE II.

Preparation of vaccine	Average duration of infectivity at different temperatures				
	60° C. Minutes	37° C. Days	10—15° C. Days	4° C. Days	- 4° C. Days
Glycerinated	11	12	203	364	> 364
Chloroformed	11.5	13	224	364	> 364
Exudate	16	20	322	> 364	> 364
Lanolinated	39	20	301	> 364	> 364
Dry powdered	51	> 20	252	287	301

I am indebted to Dr C. J. Martin for pointing out to me that the results in Table II indicate a certain regularity in the effect of temperature upon the durability of calf lymph. For example if logarithms of times as ordinates be plotted against degrees of temperature as abscissae, the results of the experiments form points on a straight line (Fig. 1).

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In other words there exists a regular logarithmic relation between the duration of infectivity and the temperature at which the lymph is kept, which is expressed by the equation

$$\frac{1}{T_2 - T_1} \log \frac{t_1}{t_2} = \text{a constant},$$

where t_1 and t_2 represent the time of durability of lymph at temperatures T_1 and T_2 respectively.

From the above observations the temperature coefficient for the velocity with which glycerinated lymph deteriorates can be determined and is found to be about 3 for each 10° , so that lymph kept at 20° C. deteriorates three times as quickly as lymph kept at 10° C.

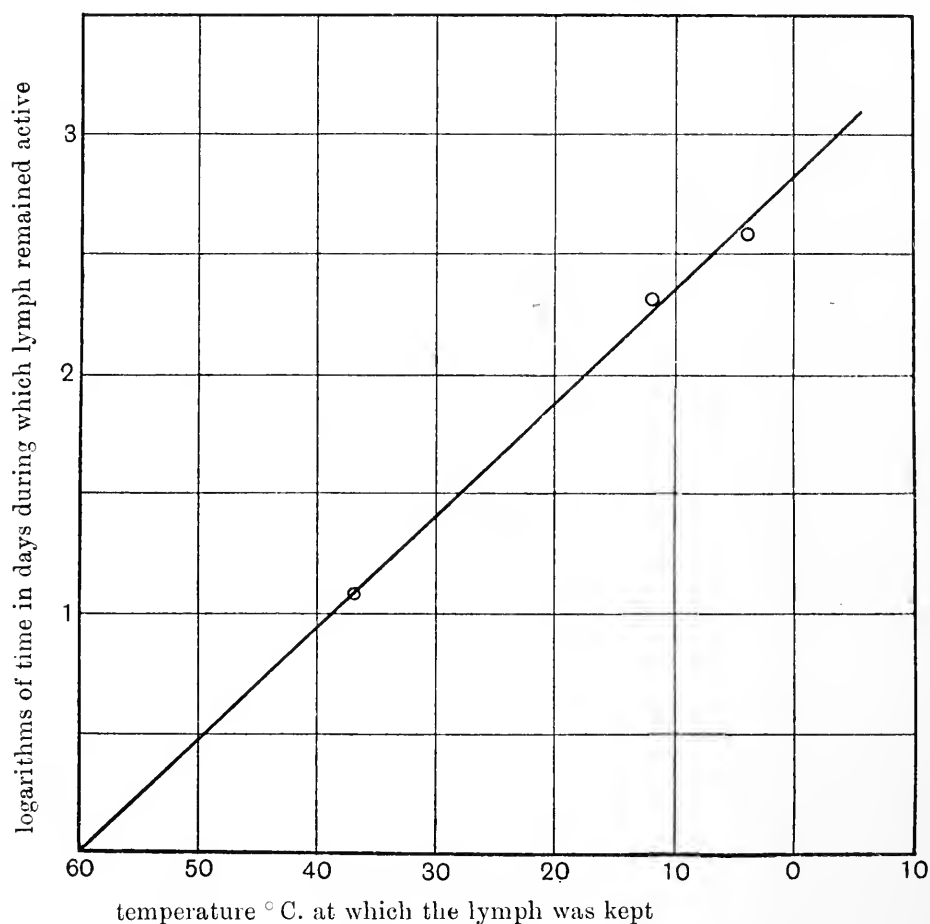


Fig. 1.

A point of interest is that the effect of temperature in hastening the deterioration of glycerinated lymph obeys the same law as is followed by an ordinary chemical reaction and the effect is moreover quantitatively of the same order, viz. two to three times for a rise of 10° C. A similar

law for the influence of temperature upon the germicidal action of mercuric chloride and other disinfectants has been found by Madsen and Nyman (1907) and H. Chick (1908) to apply in the case of anthrax spores and vegetative forms of bacteria.

This logarithmic law is obeyed up to the temperature of 37°C . If however from the data below and at this temperature, the time of durability at 60°C . is calculated from the above formula, it is found to be about one day, or one hundred times that found by experiment. Somewhere between 37° and 60° temperature *per se* begins to influence the vitality of the lymph unfavourably. If Fig. 1 is reconstructed, plotting logarithms of velocities of destruction of lymph (reciprocals of durability) as ordinates against temperature as abscissae, the points obtained for temperatures 4°C ., 12.5°C ., 37°C . lie nearly on a straight line, but the point corresponding to the temperature of 60°C . is reached by making the slope much steeper. This indicates that an added effect due to temperature alone, is, somewhere between 37°C . and 60°C ., superposed upon the gradual loss of vitality which proceeds at all temperatures.

A practical advantage which follows from the discovery of this regular temperature coefficient for this process of destruction of the virus in calf lymph is that one can foretell the results of experiments which are in themselves very tedious to make. For example the length of durability at -4°C . was found to exceed one year but was not determined in any instance (see Tables I and II), owing to the extreme slowness of the process of destruction at that temperature. By an application of the above formula it appears that at a temperature of about -4°C . glycerinated lymph would still be active for nearly three years (1000 days). This figure can be obtained also by continuing the straight line drawn in Fig. 1, until the logarithm of the corresponding time can be read off for a temperature of -4°C . (log time at this point = about 3.00 and time = 1000).

In some further experiments portions of lymph pulp derived from six calves 120 hours after vaccination were dried, powdered and sealed up in glass capillary tubes. In order to ascertain the limit of resistance of the potency of dried vaccine to a temperature of 99° to 100°C ., these capillary tubes were immersed in boiling water, one tube of each of the six experimental lymphs being kept at room temperature as a control. A few hours after such exposure these lymphs were inoculated on a calf, and the results noted 120 hours later.

These results are shown in the following table III.

TABLE III.

+ = Vesiculation. ? = Specific reaction not amounting to vesiculation.
 - = Absence of vesiculation.

Number of experiment	Potency of control	Potency of dried powdered calf vaccine in sealed capillary tubes after immersion in boiling water									
		Minutes									
		1	2	3	4	5	10	15	20	30	60
1	+	+	+	+	+	+	?	-	-	-	-
2	+	+	+	+	+	+	-	-	-	-	-
3	+	+	+	+	+	+	-	-	-	-	-
4	+	+	+	+	+	+	-	-	-	-	-
5	+	+	+	+	+	+	+	?	?	-	-
6	+	+	+	+	+	+	?	-	-	-	-

That is to say, dried powdered calf vaccine after exposure in the manner described to a temperature of 99° to 100° C. gave rise to vesiculation in every case after an exposure of five minutes, in one case after exposure for 10 minutes, and in one case showed distinct signs of specific reaction by marked induration and redness along the line of inoculation after exposure for 20 minutes.

Expressed calf lymph and lanolinated calf lymph have shown no signs of potency after exposure for one minute to a temperature of 99° to 100° C.

This resistance of dried calf vaccine to a temperature of 99° to 100° C. has apparently not been investigated before, and I am indebted to Dr C. J. Martin for suggesting it.

2. OSMOTIC PRESSURE.

Negative osmosis.

It is well known that the specific virus of vaccinia is able to withstand for many weeks association with either pure glycerine or with a solution of glycerine in distilled water in various proportions.

The result of such association is very slight progressive loss of potency on the part of the specific virus.

Similarly, association of calf vaccine with a saturated watery solution of some of the sugars, notably cane sugar, results in but slight loss of potency to the vaccine.

From some experiments which I have made, this resistance is shown to be as marked at room temperature when freshly expressed fluid vaccine is admixed with either glycerine or with a saturated watery

solution of cane sugar, as when calf pulp is admixed with these substances.

This persistence of pathogenicity implies marked ability of the vaccine virus to withstand negative osmotic pressure. For one of the chief characteristics of both glycerine and of cane sugar is their hygroscopic influence.

Positive osmosis.

Novy and Knapp (1906) found that *Tr. brucei* and *Tr. lewisi* and *Sp. obermeieri* in rats' blood were affected injuriously in varying degrees by dialysis against running distilled water for different lengths of time, extending from a few minutes to several hours.

Following on Novy and Knapp's work Ballah (1906) described some experiments on calf vaccine and concluded that the potency of vaccine was not affected by dialysis against distilled water for 18 hours. I have been able to confirm Ballah's statements.

Ballah's work, however, differed in technique from that of Novy and Knapp in an important particular, on which Ballah makes no comment. For, whereas Novy and Knapp placed blood containing their organisms in the dialyser, Ballah dialysed "vaccine emulsion." If this emulsion was a glycerinated suspension, as must be concluded in the absence of a definite statement to the contrary, then Ballah probably subjected vaccine organisms to positive osmotic pressure after they had been already subjected to negative osmotic pressure by glycerine.

It was with a view to the avoidance of such a complication that the lymph of the present series of experiments were selected in a condition as analogous as possible to the bloods of Novy and Knapp.

In the present series vaccine lymph was expressed from the vesicles of a calf 120 hours after vaccination.

This lymph was collected in glass capillary tubes which were then sealed, and coagulation was then allowed to occur. After the coagulum had shrunk somewhat—20 hours after collection—the fluid contents of the tubes were removed to fine collodion sacks. These sacks were sealed, and were suspended in running distilled water for from 2 to 24 hours at room temperature, and the potency of their contents was subsequently compared on calves with that of a control (undialysed) portion of each vaccine.

Out of a series of 37 experiments no evidence was obtained to show that dialysis had any injurious action on the specific vaccine virus.

3. FILTRATION.

During the past few years renewed attempts have been made to investigate the filterability of calf vaccine. Earlier efforts pointed to the inability of the vaccine virus to pass through bacteriological filters. But in 1905 Negri published an article which indicated that, under certain conditions, the virus was capable of passage through a Berkefeld filter. The chief difference between the technique of Negri and that of former investigators lay in the fact that Negri worked with vaccine emulsion, which, after trituration to a degree of extreme fineness, had been stored in the cold for two weeks or more after preparation. The theory advanced for the filterability of vaccine organisms of such an emulsion was that this storage led to changes in the size of the organism, analogous perhaps to the formation of spores, whereby they could pass through the filter channels while the larger sized organisms could not. Previous investigators would appear to have worked with coarser and more recent emulsions.

Remlinger and Nouri (1905), and Siegel (1905), published papers in 1905 confirming Negri's results.

In 1906 Carini (1906) published his work on the subject with a carefully reasoned argument. This last named investigator appears to think that, working on the lines laid down by Negri, a very small number of specific organisms may pass through Berkefeld V and N filters. He is however careful to mention (*a*) that his experimental inoculations of these filtrates were made on animals which were used simultaneously for the routine production of vaccine, and therefore, that accidental contamination of his experimental incisions could not be absolutely excluded; and (*b*) that the character of such vesicles as he obtained was poor, and indicated inoculation with a degenerated virus. It is important to note, though Carini does not point it out, that vesicles of this type might easily result when a partly dried incision is insufficiently inoculated with normal vaccine; such a condition in fact as might be afforded were a minute quantity of vaccine from a normally vaccinated surface to be carried into another incised, but previously uninoculated, surface of the same animal.

During the last two years I have carried out a number of experiments on the filtration of calf vaccine emulsion. During the earlier part of this work I was unacquainted with Negri's procedure, but after the publication of his technique great care was taken to follow this closely in the case of over forty experiments. In many cases the filtrates were

centrifugalised and the upper layers were pipetted off to try and concentrate any organisms which might be present.

All the filters were Berkefeld's N or V. These were new filters of the smallest laboratory pattern, sterilised before use.

In order to test the filters for possible defects, emulsions were examined, previous to passage, for extraneous micro-organisms and the filtrates were examined subsequently to ascertain whether these had passed through, and if so, in what quantity.

The filtrates were inoculated in some instances on guinea-pigs and in all cases on calves. A control, or unfiltered portion of emulsion, in each instance was inoculated on the same animal. The calves were used at the same time for the routine production of vaccine.

Of the results of the inoculations on guinea-pigs every one was negative, while the controls showed specific activity.

Of the results on calves every filtrate, with two exceptions, gave a negative result; while the controls on the same animals gave well-marked vesiculation.

In the case of the first of the two exceptions distinct vesiculation showed 120 hours after inoculation. The site of the inoculation was on the inner side of the right hind leg, about 5 cm. from the ilio-inguinal fold and at right angles to it. The inoculation was a line about 5 cm. long. The site in question had been ill-chosen for an experimental inoculation, for when the calf was standing up this surface of skin was in contact with a portion of a surface of skin which was being used for routine vaccination; although when the calf was on the vaccination-table the two surfaces were separated considerably.

In this case, the possibility that this experimental incision was inoculated accidentally from the skin surface with which it came in contact, cannot be excluded.

In the case of the second exception there was no question of direct contact between the experimental incision and the normally vaccinated surface. But at the site of inoculation, 120 hours after inoculation with a filtrate of a Berkefeld V filter, there was one spot of an apparent attempt at vesiculation tailing off into a slightly raised red papule. The appearance in fact was that of very feeble specific reaction. The control of the filtrate produced well-marked vesiculation, though not of first-rate quality, on the same animal.

Apart from the proximity of the control the whole abdomen of the calf was used for routine vaccination as well as a small portion of the thorax somewhat adjacent to the site of the experimental incision,

and it is impossible therefore to exclude entirely the possibility of accidental contamination, though it is quite possible that no such contamination occurred.

The results of these experiments as a whole cannot be said to afford confirmation of Negri's work. On the other hand they appear to be parallel with Carini's results, and to show that if the specific virus of vaccinia is capable of passage through a Berkefeld V such passage is of rare occurrence, and is limited to a very small number of germs.

It has been stated earlier that my first experiments were not carried out according to Negri's technique. With these a 1 in 5 emulsion was usually filtered through a Berkefeld V or N, and occasionally through a Pasteur-Chamberland by means of a partial vacuum, without a preliminary passage through filter paper or cotton wool. A result was then commonly obtained which I have not seen noted elsewhere.

When the filtrate was examined, after negative pressure had been going on for an hour or more, small white masses of organic matter were noticed, and often in considerable amount. These masses were not uncommonly so large as to point to a defect in the filter. But *B. subtilis* and staphylococci failed to pass through the filter, while the white particles were present in the filtrate in numerous large fragments.

With fractional filtration it was noted that while all fractions might be clear for some minutes after passage, they gradually became clouded and the white precipitate appeared, the fragments of which increased in size for a time.

This precipitate appeared in the filtrates of Berkefeld's V or N, to a lesser extent in the filtrate of a Pasteur-Chamberland, and not at all in the filtrates of a Martin gelatin filter. The precipitate generally appeared least dense in the earliest fractions.

Hitherto this precipitate, separated from the bulk of the fluid filtrate by centrifugalisation, has failed to give any specific lesions on calves, while the control (unfiltered portion of the same vaccine) has yielded typical vesicles.

In view of this fact, if for no other reason, the microscopical appearance of the precipitate is of interest. I hesitate at the present time to say that any of the microscopic elements of the precipitate resemble definitely the body or bodies described as the specific cause of vaccinia by authors, although it seems to me that in a hanging drop preparation they are not unlike some published descriptions of the vaccine organism.

SUMMARY OF CONCLUSIONS.

1. Temperature influences the rate at which glycerinated lymph deteriorates in accordance with the law of Arrhenius, expressing the effect of temperature upon chemical action; the rate of deterioration increasing three times per 10° rise in temperature.

2. Dry powdered calf vaccine in sealed glass capillary tubes still gave rise to typical vesicles after exposure to a temperature of 100° C. for from 5 to 10 minutes.

3. The vaccine virus can withstand positive osmosis for 24 hours or more, and negative osmosis for 8 weeks or more, at room temperature.

4. It appears doubtful whether the vaccine virus will pass through a Berkefeld V filter even when a stored vaccine emulsion is used for filtration. If any virus does pass through it only does so exceptionally and in small quantities.

5. When a glycerinated vaccine emulsion is filtered, white flocculi frequently form in the filtrate. These are at first minute, but subsequently adhere and form a macroscopic sediment. This sediment, when inoculated on calves, does not cause vesiculation.

Microscopically these flocculi, in hanging drop preparations, resemble the vaccine bodies described by one or more observers.

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A NOTE ON THE VARIATION OF THE RATE OF DISINFECTATION WITH CHANGE IN THE CONCENTRATION OF THE DISINFECTANT.

By HERBERT EDMESTON WATSON, B.Sc. (LOND.), A.I.C.

IN a recent paper by Miss Chick on "The Laws of Disinfection¹," it was pointed out that disinfection of bacteria is strictly analogous to a chemical reaction in which individual bacteria play the part of molecules. Thus, if n be the number of bacteria present at any time t during disinfection, $-\frac{dn}{dt} = K \cdot n$, where K is a constant. Also, if K_1, K_2 are these constants for two different temperatures T_1, T_2 , $\frac{T_1 T_2}{T_1 - T_2} \log \frac{K_1}{K_2}$ is also constant, *i.e.* Arrhenius' formula for the temperature coefficient of chemical reactions holds good in the case of bacteria as well. In addition to this, it was found that the relation between the concentration of the disinfectant and the time of disinfection (that is, the time required to reduce the original number of bacteria by a given percentage) might be approximately expressed by the empirical law

$$\frac{1}{C_0 - C} \log \frac{Ct}{C_0 t_0} = \text{a constant},$$

where C is the concentration at time t .

Now it is at once evident that this last expression admits of no physical interpretation, whereas the two former ones may be deduced theoretically, and the meaning of all the constants in them is intelligible. Consequently it seemed probable that some other expression with a physical meaning might be found which would replace the empirical one, and by analogy with chemical reactions it was thought that a law of the form $c^n \cdot t = \text{a constant}$, might hold, t being the time of disinfection for concentration c of disinfectant, and n being a constant, for it may

¹ This *Journal*, vol. VIII. p. 92, 1908.

be shown that this is the relation when one molecule of one substance reacts with n molecules of a second, the latter being in great excess.

For purposes of calculation, this law may be written

$$n \cdot \log c + \log t = \text{constant},$$

that is to say, the relation between $\log c$ and $\log t$, is a linear one.

The formula was applied to Miss Chick's results, making use of a graphical method in which $\log c$ was plotted against $\log t$. In every case the result was a straight line from the slope of which n could be calculated¹.

The following tables show the nature of the constant obtained when this value of n was substituted in the formula $n \cdot \log c + \log t = \text{const.}$ The first two columns give the relative concentrations and the times of disinfection, and are taken from the most suitable experiments recorded in Miss Chick's paper. Also for the sake of comparison, the values of the constant K obtained by means of the empirical formula $\frac{1}{C_0 - C} \log \frac{Ct}{C_0 t_0}$ are given in the third column.

TABLE I.

Disinfection of B. paratyphosus by phenol. 20° C.

Parts phenol per 1000	Time taken for disinfection	'K'	$5.5 \log c + \log t$
8	45 minutes	—	6.62
7.5	75	0.39	6.69
7	105	0.31	6.67
6.5	125	0.24	6.58
6	225	0.29	6.64
5.5	440	0.29	6.71
5	690	0.33	6.68

¹ It may perhaps be here mentioned that in cases of this kind, a graphical method is greatly superior to the usual methods of calculation, provided that the quantities plotted are so chosen that the resulting curve extends well across the paper, and, if possible, approximates to a straight line. Actually in the present case, the advantage is not very great, but in formulae such as $K = \frac{1}{t - t_0} \log \frac{n_0}{n}$, employed by Madsen and Nyman, *Zeitschr. f. Hygiene*, vol. LVII. p. 388, 1907, and H. Chick, this *Journal*, vol. VIII. p. 92, 1908, to express the reaction velocity of disinfection, the calculated value of K may lead to quite erroneous results, firstly because the values t and n may be inaccurate if taken from a single experimental value, as is usually the practice, and secondly, because the effect of a given experimental error on the value of K is greatly exaggerated when $t - t_0$ is small, while the same error when $t - t_0$ is great is almost inappreciable. The magnitude of these errors is, however, at once seen from a suitably drawn curve. Consequently, before making any deductions from values calculated by means of a formula, it is always advisable to examine the graphical solution to see if they are justified.

TABLE II.

Disinfection of Staph. pyogenes aureus by phenol. 20° C.

Parts phenol per 1000	Time taken for disinfection	'K'	$5.5 \log c + \log t$
14	4.5 minutes	—	6.95
12.5	2.5	—	6.42
10	25	0.15	6.90
8	95	0.16	6.94
7	186	0.20	6.92
6	395	0.20	6.88
4	1425	0.19	6.47

TABLE III.

*Disinfection of Anthrax spores by mercuric chloride. 18° C.
(Krönig and Paul.)*

Relative concentration of Hg ⁺⁺ ions	Time taken for disinfection	'K'	$4.9 \log c + \log t$
88.5	4.54 minutes	—	10.20
83.0	7.14	0.031	10.26
76.8	12.5	0.032	10.35
69.0	14.4	0.023	10.19
61.0	38.4	0.028	10.33

TABLE IV.

Disinfection of B. paratyphosus by mercuric chloride. 20° C.

Relative concentration of Hg ⁺⁺ ions	Time taken for disinfection	'K'	$3.8 \log c + \log t$
63	1.5 minutes	—	7.02
57.5	7	—	7.54
42.5	13	0.037	7.31
37	10	0.023	6.95
23	65	0.030	6.99
16.5	230	0.027	6.98

TABLE V.

Disinfection of B. paratyphosus by silver nitrate. 20° C.

Concentration of AgNO ₃	Time taken for disinfection	'K'	$0.86 \log c + \log t$
5000	0.75 minutes	—	3.06
1000	1.5	0.10	2.76
500	2.5	0.11	2.70
100	6.5	0.15	2.53
50	22.5	0.10	2.81
10	56	0.16	2.61
5	140	0.14	2.75
1	> 390	—	> 2.59

From these figures it will readily be seen that the constant obtained is as good as may be expected when the experimental difficulties are considered, and it may be quite well assumed that for practical purposes, the law $c^nt = \text{constant}$, is true, since it agrees within experimental error with results obtained by the best methods at present known.

It will be seen that in the above tables, n is assigned the following values: 5.5 for phenol, 3.8 for mercuric chloride, and 0.86 for silver nitrate. In all cases the bacteria disinfected are *B. paratyphosus*. The figure for disinfectant 'A' is probably about 8.5, but measurements with this substance are very difficult, and the present experimental data insufficient for more accurate deductions. These numbers are probably constant only at a constant temperature, and may vary with the bacteria disinfected, though the figures for phenol in the cases of *B. paratyphosus* and *Staph. pyogenes aureus* are the same. Information on this point would be interesting, but unfortunately is not at present available. However, from the results already obtained, it does seem possible to derive a little insight into the process of disinfection, and perhaps the following idea is not wholly unreasonable.

Each bacterium is composed of a number of molecular groups, and some or all of these contain a chemical compound which can react chemically with, say, N molecules of poison. When disinfection begins, this substance will start to react. Now, although it is capable of reacting with N molecules of poison altogether, it is evident that the substance, which we may denote briefly by the symbol X , will react initially with a smaller number of molecules and thereby form various compounds. Thus, after a short period of disinfection, there will exist in the bacterium a certain number of molecules of X which have not reacted at all, some molecules of X combined with one molecule of poison, some of X combined with two molecules of poison, and so on up to N . We must suppose however that when X has combined with more than a certain definite number of molecules of poison, it becomes incapable of performing its original function in the bacterium. Thus, for example, the compound $X + 3$ molecules of poison, may be able to play its part in the life processes of the bacterium, while the compound $X + 4$ molecules of poison may not; or perhaps the union of X with only one molecule of poison may be sufficient to destroy its power of reproduction (the criterion of vitality determined in the experiments). In any case, however, the result is the same. Disinfection will proceed gradually until a certain number of molecules, and hence of molecular groups, are, so to speak, incapacitated, and when this number reaches

some definite percentage of the total number existing in the bacterium, the whole bacterium will lose its vitality, or at any rate it will no longer grow. This occurrence enables us to measure the rate of the reaction, for the death of one bacterium indicates that a certain fixed proportion of the molecules of the substance X which it contains has reacted to a definite degree.

The question which now arises is—What is the actual meaning of n as measured above? It has already been mentioned that if the reaction under discussion were an ordinary complete chemical reaction, n would represent the number of molecules of one substance acting with one molecule of another, but the present case is rather different. For instance, suppose the bacterium ceases to grow when 70 % of the molecules of the substance X which it contains have each combined with at least two molecules of poison. When death occurs, there will be 30 % of molecules uncombined or in combination with only one molecule of poison, while 70 % will be combined with two or more molecules, and consequently the reaction is not completed at this stage, although it is the time at which n is measured. Consequently n does not in this case represent the total number of molecules of poison which can combine with one molecule of X , but the average number which have combined when the bacteria become incapable of further growth.

In this connection it is interesting to note the high value of n . The 'order of the reaction' is at least the integer next greater than $n + 1$, and so in the case of phenol we have a reaction of the seventh order at least. This result may at first sight seem rather extraordinary, especially when it is remembered that the ordinary chemical reactions which have so far been worked out, are rarely of an order greater than the third, but it must be remembered that in the case of bacterial proteins we are dealing with substances of exceedingly high molecular weight, and there seems to be no reason why such molecules should not react with a great many molecules of another less complex substance.

In the whole of the above it has been assumed that there is only one kind of active substance in the bacterium, but, of course, all that has been said applies equally well however many such substances exist, and unfortunately no clue is to be obtained by this method as to their number or nature.

Finally, a word may be said with regard to practical applications of the formula given above. These may perhaps be best illustrated by two examples.

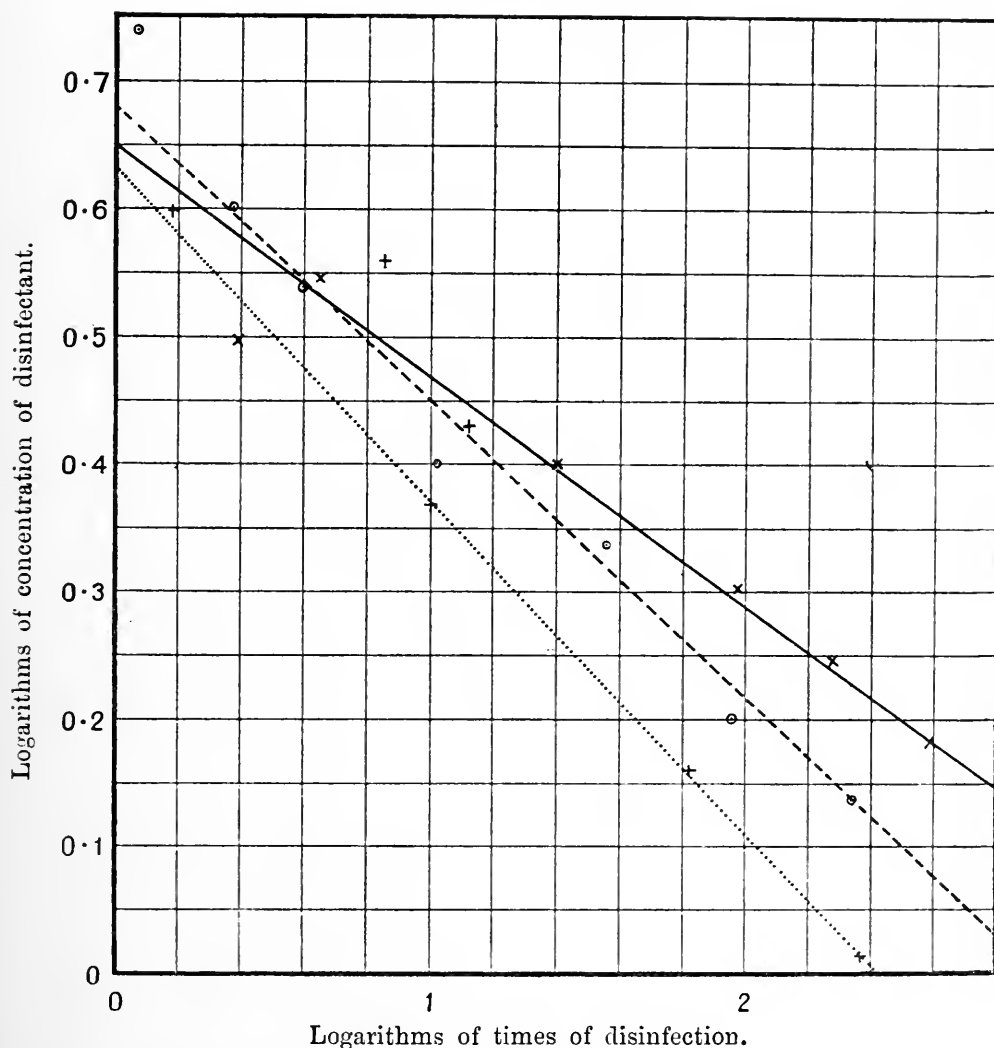
1. A solution of phenol containing 10 parts per 1000, disinfects a culture of *B. paratyphosus* in 25 minutes, another solution takes 35 minutes. What is the strength of the second solution?

Let the strength of the second solution be x .

For phenol $n = 5.5$,

therefore, $5.5 \log 10 + \log 25 = \text{const.} = 5.5 \log x + \log 35$.

From this $x = 9.4$.



The above figure shows graphically the results contained in Tables II, IV, and V, the corresponding points being denoted by the signs \times , $+$ and o respectively. The straight line drawn in full represents the variations in the case of phenol, the dotted line those of mercuric chloride, and the broken line those of silver nitrate. In order to represent all three lines on the same diagram a constant quantity has been added to or subtracted from some of the values of the logarithms as deduced from the tables. This, of course, does not alter the slope of the lines. In the case of silver nitrate the vertical scale is only one fifth of that in the other two cases, and therefore the true slope is five times as steep as it appears to be.

2. A disinfectant takes 9 minutes to disinfect a given culture when the concentration is 7 parts per 10,000 and 50 minutes when the concentration is 5 parts per 10,000. What concentration would disinfect in 15 minutes?

This is most simply solved graphically. Logarithms of the quantities given are taken and marked on a diagram where concentration is plotted against time. The two points thus obtained are joined by a straight line, and the point where this cuts the line representing time 15 minutes, shows the logarithm of the required concentration. The result thus obtained is 5.7.

In conclusion I should like to express my most hearty thanks to Miss Chick for allowing me to use her results, and also for much detailed information concerning them.

BACTERIOLOGICAL OBSERVATIONS ON COLON BACILLI
INFECTING THE URINARY TRACT, WITH SPECIAL
REMARKS ON CERTAIN COLON BACILLI OF THE
"ANAEROGENES" CLASS.

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WHILST the studies of A. C. Houston and MacConkey have extended our knowledge of the characters of the bacilli found in faeces, and those of Dudgeon and Sargent have done the same with regard to colon bacilli met with in cases of appendicitis, the fermentative activities of pathogenic colon bacilli occurring in urine have not been investigated to any great extent.

In this paper I give the results obtained by the investigation of 50 colon bacilli which were isolated from the urine of patients suffering from such conditions as cystitis and pyelitis and which appeared to be responsible for the patients' illness.

Most of the organisms isolated would be classed in the *B. coli communis* group, but six (of which two were isolated by Dr Mair) differed strikingly from the others in the fact that they produced no gas in media containing glucose. With the characters of these latter bacilli I shall deal in the second part of this paper, in the meantime confining my remarks to the gas-producers.

PART I.

Character of the Urine.

In most of the cases the urine was turbid, a deposit of pus cells occurring on standing. The reaction was almost invariably acid; in one case it was alkaline although the *B. coli* was the only organism present in it.

Microscopic examination of the deposit showed the presence of pus cells and colon bacilli, the latter being almost invariably extra-cellular.

In most cases the colon bacilli appeared as short rods or threads, but in some cases dense feltworks of long filaments were found. The assumption of the filamentous form by the bacteria was doubtless due to the action of the urea and other salts present, since, as was shown by me in a former paper, the addition of urea to a medium has this effect on the colon bacilli grown on it.

Characters of organisms.

All these organisms had the following characters in common—short Gram-negative non-sporing bacilli with rounded ends—greyish-white layer of growth on agar—gelatine never liquefied. Motility was tested for in hanging drops made from 18 hours old agar and peptone water cultures with the result that only six out of 44 were found to exhibit movement of transposition; many exhibited active Brownian movement. This absence of motility is rather remarkable and perhaps if special methods had been adopted a larger percentage of positive results would have been obtained. We may remark in passing that Dudgeon and Sargent found motility present in only 19 out of 74 colon bacilli isolated from cases of peritonitis.

Fermentative reactions produced by 44 gas-producing urinary bacilli.

The action of the bacilli on the following substances was investigated—glucose, lactose, saccharose, dulcitol and mannitol. One per cent. of these substances was added to peptone water tinted with litmus solution, and the medium distributed into Durham's fermentation tubes and sterilised in the steamer for ten minutes on each of two days, great care being taken not to overheat the medium.

It is evident from Table I that according to their action on these substances these organisms may be divided into seven groups.

TABLE I.

No. of organisms	Glucose	Lactose	Saccharose	Dulcitol	Mannitol
14	+	+	+	+	+
19	+	+	-	+	+
4	+	+	+	-	+
2	+	+	-	-	+
2	+	-	+	+	+
2	+	-	-	+	+
1	+	-	-	-	+

In this Table + indicates formation of acid and gas.

" " - " no change in medium.

It will be observed that glucose and mannitol were fermented by all of these organisms, that 39 of them fermented lactose, that 37 of them fermented dulcitol and 20 of them fermented saccharose.

MacConkey classifies lactose fermenting colon organisms into four groups according to their action on saccharose and dulcitol. Of the above organisms we have 39 lactose fermenters and the action of these on saccharose and dulcitol is seen in the following table.

TABLE II.

MacConkey's group	Organisms belonging to this group according to MacConkey	Saccharose	Dulcitol	No. of urinary bacilli belonging to each group
Group I.	<i>B. acidi lactici</i> (Hüppe)	-	-	2
Group II.	<i>B. coli communis</i> (Escherich)	-	+	19
Group III.	<i>B. neapolitanus</i>			
	<i>B. pneumoniae</i> (Friedländer)	+	+	14
	<i>B. coli communior</i> (Durham)			
Group IV.	<i>B. lactis aerogenes</i>	+	-	4
	<i>B. cloacae</i> , etc.			

With regard to certain other characters it may be mentioned that 38 out of the 44 produced fluorescence in neutral red glucose agar shake cultures, 30 caused clotting in litmus milk, 39 produced much indol, three a trace, and two no indol.

On the Drigalski-Conradi medium the growth of some of the organisms had a peculiar jelly-like appearance, especially well marked at the edge of the line of growth.

Route by which the colon bacillus enters the urinary tract.

With regard to the manner in which the colon bacillus reaches the kidneys and bladder we are unable to dogmatise, but we favour the view that it is absorbed from the intestine through the blood capillaries or lymphatics.

The fact that cases of "coli" cystitis are commoner in women than in men has been held to support the view that the colon bacilli reach the urinary system from without, the shorter urethra in the female facilitating their entrance. But the explanation may be that women suffer more from constipation than men and that therefore there is the greater liability of the colon bacilli being absorbed from the intestine. Notes of a case in which the bacilli seem to have entered from the intestine as the result of constipation and to have been excreted by the kidney without any urinary symptoms arising, may be cited here.

A woman aet. 22 was admitted into one of Professor Lindsay's Wards, Royal Victoria Hospital, Belfast, with a history of malaise of over a week's duration. Her temperature was 103·5, pulse 114; she had also the flushed face, severe frontal headache and distended abdomen characteristic of typhoid fever. Her blood gave a negative Widal reaction with the *Bacillus typhosus*. It was found that she had been completely constipated for a fortnight. On the day after admission as the result of an enema her temperature dropped two degrees. On the following day, after a second enema, her temperature became normal and continued normal. The interesting feature of this case was that during the time the girl was in Hospital, she was excreting large numbers of *B. coli communis* in her urine although she had no symptoms of kidney or bladder trouble. The spleen was palpable and on a certain day she had a dozen typical typhoid spots on the abdomen. Her blood continued to be negative to the *Bacillus typhosus* and it had no agglutinative action on the colon bacillus isolated from her urine.

This was probably a case of "faecal fever" a condition for the occurrence of which Nothnagel cites reputable authorities.

It is possible that in many individuals colon bacilli are absorbed from the intestines and excreted in the urine and that it is only in predisposed individuals that pyelitis or cystitis results.

Dudgeon found that two out of a total of 20 habitually constipated persons examined, had colon bacilli in their urine.

Agglutination of colon bacilli.

The blood serum of a small number of the cases was investigated with regard to its agglutinative action on the bacilli isolated from their urine. In most cases the result was negative even in as low dilutions as 1 in 14. In one case there was agglutination in a dilution of 1 in 50, but it was found that four out of five normal bloods also clumped this bacillus in a dilution of 1 in 50.

Further experiments convinced me that in ordinary cases of colon cystitis there is little or no increase of agglutinins in the blood of the patients. "Clumping" occurring in comparatively high dilutions is more often due to the easily agglutinable nature of the particular strain of colon bacillus used than to any absolute increase of agglutinins in patient's blood.

PART II.

Bacilli of the "anaerogenes" class.

Of the six bacilli which formed no gas (anaerogenic) in glucose media one differed from the others in being utterly devoid of fermentative action on carbohydrates and in causing liquefaction of gelatine. It was associated with a non-lactose fermenting colon bacillus. That it was probably infecting the patient seemed to be indicated by the fact that the patient's blood in 1 in 50 and in 1 in 100 dilutions agglutinated it in one hour whilst normal blood under the same conditions had no effect on it. Moreover it was isolated on two occasions, an interval of two months elapsing between the examinations. The remaining five are members of the same group, but before considering their characters in detail we will preface our remarks with a short account of our knowledge of this class of micro-organisms.

Lembke in the faeces of a dog found a colon-like bacillus which was non-motile, produced indol, acidified and clotted milk but produced only acid in glucose and lactose bouillon. To this organism he gave the name *Bacillus coli anaerogenes*.

Mair isolated two organisms of this class from cases of cystitis and described the cultural characters of one of them. I have obtained cultures from Mair of his bacilli and have compared them with the three isolated by myself.

Dudgeon found a pathogenic organism of this class in an enlarged prostate gland. He gave a careful description of his bacillus and alluded to the fact that A. C. Houston had described somewhat similar organisms.

Cathcart described such bacilli among the flora of "blown" tins of preserved food.

Castellani in four cases of continued fever occurring in Ceylon found the causative organisms to belong to this class.

It would be perhaps well to give some such distinctive name as the *B. coli anaerogenes* to this group of micro-organisms. The term "coli"

would indicate not only their relationship to the *B. coli communis* but also the habitat of some at least of them, whilst the term anaerogenes would serve to distinguish them from the intermediate typhoid-colon gas-producing group to which such organisms as the paratyphoid bacilli, Gaertner's bacillus and the Aertryck bacillus, belong.

Characters of the Belfast anaerogenic bacilli.

The Belfast anaerogenic bacilli had the following characters in common:—Gram-negative, non-sporing bacilli—greyish-white growth on agar—uniform turbidity in broth—no liquefaction of gelatine—abundant red growth on the Drigalski-Conradi medium. The fermentative action of the Belfast bacilli was investigated and the result is given in Table III along with certain other characters of the organisms. For contrast we give the corresponding properties possessed by the *B. typhosus*.

We may observe that the fermentative changes produced were very constant.

An analysis of Table III shows:

- (1) that two of the cultures were feebly motile, the other three being non-motile,
- (2) that all five produced acid but no gas from glucose, laevulose, lactose, arabinose and rhamnose,
- (3) that none of them had any action on inulin, erythrite and adonite,
- (4) that all five fermented mannite but that *Belfastiensis I* differed from the others in producing not only acid but gas also,
- (5) that they differed from each other in their respective capacities for fermenting such substances as maltose, saccharose, glycol, glycerine, dulcitol, salicin, sorbit and dextrin,
- (6) that no two of the five were identical in their fermentative characters,
- (7) that four of the five produced much indol, the fifth being negative in this respect,
- (8) that all five produced permanent acidity in litmus milk: three of them also caused clotting,
- (9) that none of them produced gas or fluorescence in glucose neutral red agar shake cultures,
- (10) that in their growth on potato and gelatine some possessed colon-like, others typhoidal characters.

TABLE III.

Name of organism	Motility	Glucose	Laevulose	Maltose	Lactose	Saccharose	Dulcitol	Mannitol	Glycol	Glycerine	Erythritol	Adonitol	Arabinose	Raffinose	Salicin	Sorbitol	Rhamnose	Dextrin	Inulin	Litmus milk		Indol	Glucose neutral, shake cultures	Potato	Surface colonies on gelatine plates	
<i>Belfastiensis I.</i> (Mair)	-	Ac	Ac	Ac	Ac	-	-	AcG	-	-	-	-	Ac	AcG	Ac	Ac	Ac	Ac	-	-	+	+	+	no gas, no fluorescence	brown growth	Transparent "vine-leaf" colony
<i>Belfastiensis II.</i> (Mair)	+	Ac	Ac	Ac	Ac	Ac	-	Ac	-	Ac	-	-	Ac	Ac	-	Ac	Ac	-	-	+	+	+	„	moist invisible growth	Brownish colonies, short outrunning branches of growth	
<i>Belfastiensis III.</i> (Wilson)	-	Ac	Ac	Ac	Ac	-	Ac	Ac	Ac	Ac	-	-	Ac	-	-	Ac	Ac	Ac	Ac	-	+	+	„	brown growth	Faintly brownish circular colonies, even edge	
<i>Belfastiensis IV.</i> (Wilson)	-	Ac	Ac	-	Ac	-	-	Ac	-	Ac	-	-	Ac	Ac	Ac	Ac	Ac	-	-	+	+	+	„	moist invisible growth	Brownish colonies, short outrunning branches of growth	
<i>Belfastiensis V.</i>	+	Ac	Ac	Ac	Ac	Ac	-	Ac	-	-	-	-	Ac	Ac	Ac	-	Ac	Ac	-	+	+	+	„	brown growth	Brownish colonies, slightly crenated margin	
<i>B. typhosus</i>	+	Ac	Ac	Ac	-	-	-	Ac	-	..	-	-	-	-	-	Ac	-	Ac	Ac	-	+	-	„	moist invisible growth	Translucent "vine-leaf" colonies	

In this table Ac indicates production of acid.
Ac G " " " and gas.
- " " a negative result.

TABLE IV.

Name of organism	Motility	Bouillon	Glucose	Maltose	Saccharose	Lactose	Raffinose	Arabinose	Mannite	Dulcit	Salicin	Dextrin	Sorbit	Litmus milk	Indol	Neutral red shake cultures
<i>B. coli communis</i>	+	G T	Ac G	Ac G	-	Ac G	Ac G	Ac G	Ac G	Ac G	Ac G	Ac G	Ac G	Ac C	+	fluorescence
<i>B. enteritidis</i> (Gaertner)	+	G T	Ac G	Ac G	-	-	-	Ac G	Ac G	Ac G	-	Ac G	Ac G	Ac then alk.	-	„
<i>B. Paratyphosus B.</i>	+	G T	Ac G	Ac G	-	-	-	Ac G	Ac G	Ac G	-	Ac G	Ac G	Ac then alk.	-	„
<i>B. Paratyphosus A.</i>	+	G T	Ac G	Ac G	-	-	-	Ac G	Ac G	Ac G	-	Ac G	Ac G	Ac	-	„
<i>B. Belfastiensis III.</i>	-	G T	Ac	Ac	-	Ac	-	Ac	Ac	Ac	-	Ac	Ac	Ac C	+	no fluorescence

TABLE V.

Name of organism	Motility	Bouillon	Glucose	Mannite	Lactose	Saccharose	Dulcite	Litmus milk	Indol
<i>B. pyogenes foetidus</i>	-	G T	Ac	Ac	Ac	Ac	Ac	Ac C	+
<i>B. ceylanensis B.</i> (Castellani)	-	G T P	Ac	Ac	Ac	Ac	Ac	Ac C	+
Cathcart's bacilli, <i>g</i>	Ac	Ac	Ac	Ac	-	Ac	+
<i>f</i>	Ac	Ac	-	Ac	-	Ac C	trace
<i>b</i>	Ac	Ac	Ac	Ac	-	Ac C	trace
<i>c</i>	Ac	-	Ac	-	-	Ac C	trace
VI.	Ac	Ac	-	-	-	Ac	+
VIII.	Ac	Ac G	-	-	-	Ac C	+
Dudgeon's bacillus	+	G T	Ac	Ac	Ac	-	-	Ac	+
<i>Belfastiensis I.</i>	-	G T	Ac	Ac G	Ac	-	-	Ac C	+
II.	+	G T	Ac	Ac	Ac	Ac	-	Ac	+
III.	-	G T	Ac	Ac	Ac	-	Ac	Ac C	+
IV.	-	G T	Ac	Ac	Ac	-	-	Ac	+
V.	+	G T	Ac	Ac	Ac	Ac	-	Ac C	-
<i>B. ceylanensis A.</i> (Castellani)	-	G T P	Ac	-	-	-	-	Ac C	-
<i>B. typhosus</i>	+	G T	Ac	Ac	-	-	-	Ac	-
<i>B. dysenteriae</i> (Flexner)	-	G T	Ac	Ac	-	-	-	Ac then alkaline	-
<i>B. dysenteriae</i> (Shiga)	-	G T	Ac	-	-	-	-	Ac then slightly alkaline	-

In these tables + indicates a positive reaction.

- indicates a negative reaction.

Ac indicates production of acid.

Ac G indicates production of acid and gas.

Ac C indicates production of acid and clot.

G T indicates general turbidity

G T P indicates general turbidity and formation of a pellicle.

In Table IV we have compared the fermentative changes produced by certain well-known intestinal organisms with those produced by *Bacillus Belfastiensis* III.

And in Table V from the data at our disposal we have contrasted the Belfast organisms with those of Castellani, Dudgeon, and Cathcart as well as with the *B. pyogenes foetidus*, the *B. dysenteriae* and the *B. typhosus*.

A glance at Table V shows the great amount of variation there is amongst micro-organisms of this class.

Belfastiensis I (Mair) and Cathcart's bacillus VIII resemble each other in forming acid and gas in mannite broth.

One notes a progressive decrease in fermentative activity in passing down the Table, the *B. pyogenes foetidus* and *B. ceylanensis* *B.* fermenting all the sugars and alcohols in the list, whilst the *B. dysenteriae* of Shiga is able to ferment glucose only.

Dudgeon's bacillus and *B. Belfastiensis* IV give identical fermentative reactions with the substances mentioned in the Table.

B. Belfastiensis IV, *B. Belfastiensis* V and Cathcart's bacillus *b* differ from each other in regard to indol formation and clotting of milk but in other respects resemble each other.

We thus see that these "anaerogenic" colon bacilli have characters in which they resemble the *B. coli communis*; such are the feeble motility, the fermentation of lactose, the production of indol, the formation of acid and clot in litmus milk, the brown growth on potato. On the other hand in their absence of gas forming powers they resemble the *Bacillus typhosus* and the *B. dysenteriae*.

Belfastiensis I in producing gas from mannite and sorbit forms a connecting link between the gas-producing (aerogenic) and the non gas-producing (anaerogenic) bacilli.

The patients from whom these anaerogenic bacilli were isolated presented on four occasions the signs and symptoms of cystitis, in the fifth case those of pyelitis.

In three of the cases the bacilli were isolated on two occasions, an interval of two months elapsing between the examinations.

In all cases the bacilli were obtained in practically pure culture.

In conclusion I desire to acknowledge my indebtedness to Professor Symmers for affording me every facility for carrying out this investigation in his Laboratory, and also to those medical gentlemen who supplied me with the necessary material.

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MAIR, W. *Brit. Med. Journ.* Ap. 21, 1906, p. 908.
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ABSTRACTS OF OFFICIAL PUBLICATIONS, ETC.

Many investigations relating to Hygiene and Public Health, possessing permanent scientific value, appear in Annual Reports of Medical Officers of Health, Blue Books and other Publications of Official Bodies within the British Empire. These are not easily available and as a rule no record of them appears in scientific journals or in an accessible form ; consequently much valuable work is lost sight of. To obviate this difficulty the Editors of the "Journal of Hygiene" propose to publish from time to time abstracts of such publications, or parts of them, as appear of sufficient permanent interest. They will be glad to receive copies of official Reports coming under the above description.

THE CAMPAIGN AGAINST ANKYLOSTOMIASIS IN PORTO RICO,

being an abstract of the "Report of the Permanent Commission for the Suppression of Uncinariasis in Porto Rico for the Fiscal Year 1906—1907" (San Juan, Sept. 30, 1907).

THE widespread prevalence of ankylostomiasis in Porto Rico and the enormous amount of illness thereby produced was fully realised by the authorities some five years since, and the possibility of treating the cases wholesale with success was demonstrated by the admirable work of Ashford, King and Igaravidez (see this *Journal*, vol. VI. 1906, p. 656). As the outcome of these enquiries, there was appointed in 1906 a Permanent Commission for the Suppression of Uncinariasis in Porto Rico, consisting of Drs P. G. Igaravidez (Chairman), I. G. Martinez and F. Sein y Sein, with a grant for the year of fifty thousand dollars. The present report gives an account of the work done in the year ending 30th June, 1907. The methods adopted were much the same as before. In addition to a central station in charge of the chairman, 34 dispensaries were opened in different parts of the island : some of these only came into use during the latter part of the period under review. Patients attend at these stations, have their stools examined

and receive appropriate anthelmintic drugs which are in nearly all cases taken away and consumed at home. A week later the stools are re-examined, and so on till *Ankylostoma* eggs are no longer found or the patient ceases to return. During the year 89,233 patients (*i.e.* 11 per cent. of the whole population) paid 425,131 visits to the dispensaries, as well as a few who were found at the first examination to be free from infection. In 26 per cent. the clinical type of the disease was severe. The results of treatment were as follows :

Cured	22,936 = 25·7 per cent.
Practically cured	15,507 = 17·4 „
Under treatment	36,132 = 40·5 „
Ceased to return	14,451 = 16·2 „
Died	193 = 0·2 „

Under the heading "cured" are included only those cases whose faeces ceased to show eggs and whose blood was restored to a normal condition as judged by the haemoglobinometer. The "practically cured" group comprises those who had had four doses of anthelmintics, a treatment which previous experiments had shown to expel on an average 96 per cent. of the worms. Those who "ceased to return" were also doubtless much benefited by the dose received.

The Commission pin their faith on thymol and beta-naphthol as anthelmintics, using sodium sulphate as a purgative. Thymol is on the whole regarded as the more efficacious, being given as a dose of 3 to 4 grammes (45 to 60 grains) once a week. The corresponding dose of beta-naphthol used is 1·5 to 3 grammes (23 to 45 grains). Each patient receives with the medicine a card as follows :

"Anemia Commission of Porto Rico.

Take one of the two purgatives given to you to night.

Take at six o'clock to-morrow morning half the capsules.

Take the other half at eight o'clock the same morning.

Take a purgative at ten o'clock.

You should neither drink wine nor any alcoholic liquor during the time you are taking these medicines.

Come for more medicines, until the physician says you are cured.

Have a privy in your house. Do not defecate on the surface of the ground, but in the privy.

Do not walk barefooted so that you may avoid contracting mazamorra in your feet. Wear shoes, and you will never suffer from anemia."

The farm-owners are also circularised and, after pointing out the loss in working capacity which anaemia entails, are urged to stop the pollution of the soil and to introduce the use of shoes. The Commission point out that once a man has been persuaded to wear boots his feet will become more tender, and he will not be so anxious to discard them in the future.

That an enormous amount of good has already been done in restoring the working class to an efficient state of health is obvious enough. How far the campaign has diminished the infectivity of the island cannot as yet be gauged. At present there are so many larvae (whose free existence may certainly extend to more than a year) about, that some reinfection must occur. At Utuado, 998 patients who had been cured in 1904—6 returned for examination; only 42 of them had become infected again. It is of course hopeless to expect that the infection will ever be completely eradicated, but, if the Commission proceeds for a few years along the present lines, it is clear that it will be very much reduced; this annual task of treating cases would then be brought within quite moderate limits.

This admirable work has been effected without any great cost. The total expenses for the year amounted to £9,600 of which salaries accounted for £7,200 and drugs for £1,100. The dispensary buildings and some other expenses are provided by the municipalities. There are therefore no overwhelming financial reasons why a similar campaign should not be started in other places, such as Trinidad, where it is no doubt as badly needed as in Porto Rico.

Incidentally the Commission have found *A. duodenale* in Porto Rico as well as *A. americanum*, and have made a number of interesting observations on the clinical aspects of the disease and its pathology. Having infected a young guinea-pig through the skin with larvae of *A. americanum* without producing the slightest signs of irritation of the skin, they transfer the result in detail to man and conclude that mazamorra ("ground itch" or "bunches") is not due to *Ankylostoma* but to a secondary infection of the points of entrance of the larvae. That the boils and pustules which occur are due to pyogenic organisms is doubtless true, but it seems much more likely that the urticarial wheals which precede such lesions are due directly to the larvae of the worm.

A. E. B.

PUBLICATIONS RECEIVED

BOOKS.

- BROWN, W. CARNEGIE (1908). *Sprue and its treatment*. London: John Bale, Sons and Danielsson, Ltd., 83-91, Great Titchfield Street, Oxford Street, W. 259 pp., 2 pls. 21 × 17 cm. Price 6/- net. Cloth.

Students of Tropical Medicine will be much indebted to the Author for bringing together much valuable information on the subject of Sprue. The book is divided into 25 chapters dealing with the history, symptoms, morbid anatomy and pathology, diagnosis, treatment, etc., and prevention of the disease. The book is remarkably well printed and the publishers are to be congratulated upon the appearance which it presents.

- DIEUDONNÉ, A. (1908). *Immunität Schutzimpfung und Serumtherapie*. Zusammenfassende Uebersicht ueber die Immunitätslehre. 5th ed. Leipzig: Verlag von Johann Ambrosius Barth. 234 pp., 4 figs. Price 6.80 Marks (unbound). 7.80 Marks (bound).

Without pretending to be exhaustive this book will prove very useful to those desiring a short and clear summary of our present knowledge regarding immunity, protective inoculation and serum therapeutics.

- DUNCAN, A. (1908). *A Guide to Sick Nursing in the Tropics*. London: The Scientific Press, Ltd., 28 and 29, Southampton Street, Strand, W.C. 162 pp. 19 × 13 cm. Cloth.

Lectures which appeared originally in *The Nursing Mirror* are here gathered together in book form. The author deals with the cause and prevention of the diseases of the tropics and the special nursing required in different cases. Naturally the little book is elementary in character.

- FIRTH, R. H. (1908). *Military Hygiene. A Manual of Sanitation for Soldiers*. London: J. & A. Churchill, 7, Great Marlborough Street. 299 pp., 40 figs. 19 × 13 cm. Price 3/6 net. Cloth.

Lieut.-Colonel Firth's position as officer in charge of Army Sanitation at Aldershot and his having been Professor of Military Hygiene in the Royal Army Medical College, London, sufficiently indicate that he is dealing with a subject upon which he is an authority. The book is divided into twelve chapters dealing with: military morbidity and mortality; the causes of disease; the chief preventable diseases of soldiers; principles of disease prevention; organization of sanitary effort; the recruit; physical training of the soldier; the barrack; water supplies; the food of the soldier; clothing, equipment, and the work of the soldier; the march and the camp.

GRIMSHAW, J. (1908). *Your Child's Health*. London: J. & A. Churchill, 7, Great Marlborough Street. 144 pp. 21 × 14 cm. Price 2/6 net. Boards.

As indicated by the title the book is popular in character and intended as a guide to parents and others who have charge of children.

MACFADYEN, ALLAN (1908). *The Cell as the Unit of Life*, and other lectures delivered at the Royal Institution, London, 1899–1902. An introduction to biology. Edited by R. Tanner Hewlett. London: J. & A. Churchill, 7, Great Marlborough Street. 381 pp., 14 figs. 21 × 15 cm. Price 7/6 net. Bound.

The volume includes a series of lectures delivered by the late Dr MacFadyen during his tenure of the Fullerian Professorship of Physiology. A biographical notice and portrait are included in the volume which has been ably edited by the author's friend Prof. Hewlett. The lectures are well worth reading, although some years have passed since they were delivered. The volume is a fitting tribute to Dr MacFadyen's memory.

PAGLIANI, L. (1908). *Trattato di Igiene e di Sanità Pubblica* colle Applicazioni alla Ingegneria e alla Vigilanza Sanitaria. Volume I. Dei Terreni e delle Acque in rapporto colla Igiene e colla Sanità pubblica. Milano: Dottor Francesco Vallardi. 1020 pp., 450 figs. together with maps and charts. Price 30 Lire unbound.

An exhaustive work of which this, the first volume, deals with soil and water in relation to hygiene and public health. A special part is devoted to parasitic diseases which stand in direct relation to the hydrotelluric condition of the soil, malaria, etc. The methods of examining soil and water are dealt with in an Appendix. Professor Pagliani is to be congratulated on this monumental work which must have entailed great labour. His experience as late Director of Public Health for the kingdom of Italy has given him a wide experience in the practical problems of hygiene and public health. The work will assuredly meet with the warm welcome it deserves.

PORTER, C. (1908). *School Hygiene and the Laws of Health*. A text book for teachers and students in training. New edition. Longmans, Green & Co. 39, Paternoster Row, London. 335 pp., 121 figs. 19 × 13 cm. Price 3/6 net. Cloth.

This book appeared in 1st edition in 1906, so that the appearance of the 2nd edition within two years sufficiently proves that it has found appreciative readers. The new edition contains fresh material in the form of appendices and a new chapter on "Medical Inspection of Schools and School children."

PRESCOTT, S. C. and WINSLOW, C. E. A. (1908). *Elements of Water Bacteriology with special reference to Sanitary Water Analysis*. 2nd ed. New York: John Wiley & Sons. London: Chapman & Hall, Ltd. 258 pp. 19 × 13 cm. Price 6/. Cloth.

The purpose of the book is to gather together the results of American observers "in such a form as to give a correct idea of the best American practice" in bacteriological water analysis. Since we owe much to American workers on this subject the little book should be welcome to English readers desiring information on American methods.

STILES, C. W. and HASSALL, A. (1908). *Index-Catalogue of Medical and Veterinary Zoology*. Subjects: Trematoda and Trematode Diseases. Treasury Dept.,

Public Health and Marine-Hospital Service of the United States. Bulletin No. 37. 401 pp. Washington: Government Printing Office.

An exceedingly valuable catalogue.

THRESH, J. C. (1908). *A Simple Method of Water Analysis*. Especially designed for the use of Medical Officers of Health. 61 pp. 6th edition. London: J. & A. Churchill, 7, Great Marlborough Street. 17×11 cm. Price 2/6 net. Cloth.

WASIELEWSKI, TH. von (1908). *Studien und Mikrophotogramme zur Kenntnis der Pathogenen Protozoen*. Heft 2, Untersuchungen ueber Blutschmarotzer. 175 pp., 25 text figs. and 8 plates (70 photomicrographs). Leipzig: Johann Ambrosius Barth. Price 12 Marks, unbound.

The second part of Wasielewski's studies on pathogenic protozoa relates to Haematozoa and contains a good deal of original matter. There are six chapters dealing with: The distribution of haematozoa in Germany, *Plasmodium vivax* and *Laverania malariae*, *Plasmodium praecox*, the transmission of avian plasmodiosis and the course of the disease in birds, the sexual forms of *Haemoproteus* and *Leucocytozoon*. The work will be very useful to protozoologists and the many original photomicrographs are of great interest.

NEW JOURNALS.

Desinfektion Monatsschrift, Jahrg. I., Heft 1, July, 1908. 42 pp. Issued by Professors Flügge, Gaffky, Kirchner, Proskauer and Baurat Herzberg, under the Editorship of Drs Lentz and Lockemann (of the Royal Institute for Infectious Diseases, Berlin). Berlin: Deutscher Verlag f. Volkswohlfahrt. Annual Subscription 10 Marks, single numbers 1 Mark.

The opening number contains: Ueber ein neues Prüfungsverfahren von Sterilkatgut auf Keimfreiheit, by W. Hoffmann, pp. 2-12.—Ueber zwei neue Formaldehydseifenpräparate, by E. Seligmann, pp. 12-18.—Literature on Disinfection: bibliography compiled by Prof. O. Hamann (Librarian of the Royal Library in Berlin) pp. 18-23 (only recent titles).—Reviews of books and papers appearing in Journals, pp. 24-33.—Sanitary laws and regulations in Germany and abroad. New patents (described with figures). Brief survey of various questions: food preservation, etc., pp. 34-42.

Bulletin de la Commission Internationale Permanente pour l'Étude des Maladies Professionnelles. 1ère Année, No. 1, April 1908, 16 pp. Florence: Louis Niccolai.

Includes the statutes of the Commission, an account of its first meeting, a list of its members, a financial report, a list of prizes offered. An (unofficial) part deals with a review of progress in different countries, reviews of publications dealing with diseases of occupation and a bibliography of recent literature on this and allied subjects.

Bulletin of the Department of State Medicine and Public Health. Western Australia. Nos. 1-3, 6 pp.; January—March, 1908. Perth: F. W. Simpson, Government Printer.

It is intended that this shall be a regular publication in the future, its object being to disseminate information amongst Local Boards.

The Crèche. The Official Organ of the National Society of Day Nurseries. Vol. I. No. 3; April—June, 1908. Edited by Dr F. S. Toogood. Published quarterly. Price 6d. Annual subscription, post free, 2/6. London: The Specialist Press, Ltd., 5, Plough Court, Fetter Lane, E.C.

The Quarterly Journal of Medicine, Vol. I. No. 3; April, 1908. Edited by W. Osler, J. Rose Bradford, A. E. Garrod, R. Hutchison, H. D. Rolleston and W. Hale White. Oxford: at the Clarendon Press. Subscription 25/- a year; single numbers 8/6 net.

Contains the following papers: A Contribution to the Subject of Rheumatism, based upon a Study of 52 Cases in Children under Five Years of Age, and an Analysis of 100 Cases of Fatal Suppurative Pericarditis in Childhood, by F. J. Poynton, pp. 225–238.—Chloroma and Acute Lymphatic Leukaemia, with an Account of Four Cases, and a Discussion of the Pathology of the Diseases, by C. H. Treadgold. Plate 33, pp. 239–286.—The Paratyphoid Glands: Part II. Their Pathology in Man, by D. Forsyth, pp. 287–311.—Persistent Hereditary Oedema of the Legs with Acute Exacerbations. Milroy's Disease, by W. B. Hope and H. French. Plates 34 and 35, pp. 312–330. The Inheritance of Certain Human Abnormalities, by A. M. Gossage, pp. 331–348. Critical Review.—Caisson Disease, by A. E. Boycott, pp. 349–374.

VARIOUS PERIODICAL PUBLICATIONS.

Beihefte zum Archiv f. Schiffs- u. Tropenhygiene. The new "Beihefte" promise well. The following have reached us, being appended to vol. XI. of the Archiv: VIERECK, H. (1907). Studien über die in den Tropen erworbene Dysenterie. Beiheft 1, 41 pp., 3 pl. BENTMANN and GÜNTHER (1907). Beiträge zur Kenntnis des Trypanosoma gambiense. Beiheft 2, 70 pp., 2 pl. GIEMSA, G. and SCHAUMANN, H. (1907). Pharmakologische und chemisch-physiologische Studien über Chinin. Beiheft 3, 84 pp. ZIEMANN, H. (1907). Wie erobert man Afrika für die weisse und farbige Rasse? Beiheft 5, 29 pp. WERNER, H. (1907). Ueber die Nieren beim Schwarzwasserfieber. Beiheft 6, 20 pp., 3 pl.

Communications de l'Institut Sérothérapique de l'État Danois. Tome II. 1908. Contains the following papers (bound reprints): A Report of immunization curves derived from goats treated with certain haemolytic bacterial toxins (Vibriolysin and Staphylolysin), by L. W. Famulener, 15 pp., 12 curves.—On the absorption of antibodies from the subcutaneous tissues and peritoneal cavity, by J. Henderson Smith, pp. 205–215, 5 figs.—Untersuchungen über aktive und passive Immunisierung mit Vibriolysin, by T. W. Tallquist, pp. 165–193, 15 figs.—Zur Pathogenese der perniziösen Anämie, mit besonderer Berücksichtigung der Bothriocephalusanämie, by T. W. Tallquist, 106 pp.—Contributions aux études théoriques sur la Désinfection, by Th. Madsen and Max Nyman, pp. 105–126.—Ein neues Saccharimeter, by L. E. Walbum, 3 pp.—Studien über Hetero- und Isantagonismus, mit besonderer Berücksichtigung der Verhältnisse bei Infektiösen Erkrankungen der Harnwege, by R. Faltin, 42 pp., 1 fig.—Tetanusgift im Serum eines diphtherieimmunisierten Pferdes, 5 Tage vor dem Ausbruch von Tetanus, by Th. Madsen, 2 pp.—Versuche über Hämolyse, by Svante Arrhenius, 35 pp.

Studies from Institute for Medical Research, Federated Malay States. Vol. III.

Part III., 1908. Contains the following papers: Breeding Grounds of Culicidae, by C. W. Daniels.—The Culicidae of Malaya, by G. F. Leicester. Singapore: Kelly & Walsh, Ltd., Printers.

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THE TOXICOLOGY OF NICKEL CARBONYL. PART II.

Plates VII—IX, and Four Figures.

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I. INTRODUCTION.

Nickel responsible for the symptoms of Nickel Carbonyl poisoning.

IN Part I¹ it was shown that the toxic symptoms after nickel carbonyl inhalation are not due to either the nickel carbonyl as such or to the carbon monoxide which is given off when the compound is exposed to air and moisture at body temperature. It was therefore concluded that they are occasioned by the absorption of the nickel set free and that nickel carbonyl poisoning is a particular case of nickel poisoning.

The fact that the nickel is deposited over the immense surface of the lungs in a condition which is especially favourable for absorption renders a study of the toxicology of this compound of great interest.

The results of this second part of the investigation will be presented in the following order :

1. Further evidence that the nickel is responsible for the symptoms, by comparison of the course of poisoning and of the changes induced by the inhalation of nickel carbonyl vapour with the course of poisoning and changes induced by the administration of other nickel compounds.

2. An attempt to trace the nickel through the organism.

3. A comparison of the toxic effects of nickel, iron and cobalt.

¹ See *Journ. of Hygiene*, VII. 4, pp. 525—551. 1907.

II. COURSE OF POISONING IN ANIMALS.

(i) *Methods employed.*

The experiments with nickel and iron carbonyl were, save for a few of the earlier ones, in which another method which did not prove satisfactory was employed, carried out as follows:—the liquid carbonyl was placed in a graduated burette of 1 c.c. capacity (see Plate IX *e*) provided with an ordinary stopcock above and a three-way stopcock below. The lower end of the burette was passed through a rubber cork, fitted into a glass receiver (*f*) which contained a little cotton wool to catch the carbonyl. A given quantity of the carbonyl was run out of the burette by turning on the three-way stopcock. The tap having been reversed, a small quantity of carbon dioxide was allowed to pass through the receiver containing the nickel carbonyl into a large gasholder (A), the capacity of which was approximately 240 litres so as to evaporate the carbonyl in the receiver. A little air was blown into the gasholder before the carbon dioxide was turned on, to prevent the carbonyl condensing in the latter. While the carbon dioxide was blowing all the carbonyl over, air was introduced into the gasholder through the second tap (*d*). The quantity of carbonyl being known (1 c.c. of liquid nickel carbonyl yields 181.1 c.cm. of vapour at 0° C. and 760 mm. Hg., and 1 c.cm. of iron carbonyl yields 167.39 c.cm. of vapour) and the capacity of the gasholder having been determined and recorded on a scale (*c*) attached to the upright, the volume percentage of the carbonyl in the air could be accurately measured. After all the fluid had been evaporated, the taps of the gasholder were closed and the heavy weights exchanged for one of two kilograms. The carbonyl burette was removed and the chamber (B) was connected to the gasholder by applying the tube *g* to the tap *b*. The chamber was an air-tight box, made of mahogany and glass with rubber stoppings adjusted to the windows and lid. From the outlet (*h*) a tube carried off the vapour and air to a Bunsen burner, where all the carbonyl was dissociated. A screw clamp applied to this tube regulated the flow of the vapour mixture passing through the chamber. The rate was determined by timing the descent of the level of the top of the gasholder against the scale (*c*). It was found that a satisfactory rate for the purpose was three litres per minute.

The poison chamber contained air at the beginning of each experiment, and this air was gradually replaced by the vapour mixture in the

gasholder. In order to determine the rate of replacement, test experiments were conducted with oxygen (in which Dr Boycott, of Guy's Hospital, kindly assisted me) and the gas issuing from the outlet was analysed at short intervals. The first series of determinations were conducted with an inanimate object in the chamber in the place of the rabbit. The second series was conducted with a living rabbit, and it was found that the mechanical action of the respiration, etc. on the mixing of the gases was appreciable. With the rate of flow employed the quantity of oxygen absorbed by the rabbit may be neglected. It was therefore considered better to utilise the results of the second series alone, and from the figures obtained a curve of the rate of displacement was interpolated, the ordinates representing the percentage of vapour mixture which has replaced the air in the chamber and the abscissa representing the time in minutes. The figure lying above the curve has been plotted as a rectangular figure, and from this it appears

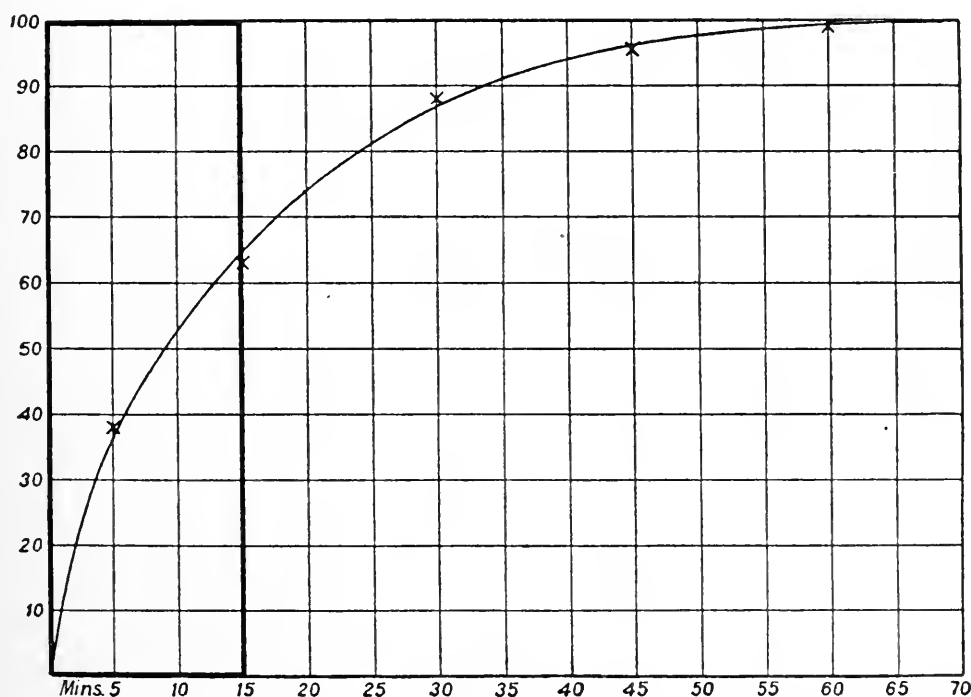


Fig. 1. Curve showing Rate of Displacement in chamber.

that a deduction of $14\frac{1}{2}$ minutes would be a necessary correction in taking the time during which the animal was in the poison chamber as indicating the opportunities for intake of nickel carbonyl vapour. The curve and rectangular figure is given in Fig. 1).

It must however be pointed out that nickel carbonyl would not behave like a stable gas, such as carbon monoxide, on account of its

ready dissociability in the presence of air and moisture at the temperature of the animal body (see Part I) and the results obtained by means of this curve are only approximate.

(ii) *Concentration of vapour and length of exposure.*

A large number of the experiments with nickel carbonyl are available for the purpose of determining how much of the vapour has to be inhaled to produce death. Naturally some individual idiosyncrasy played a considerable rôle in the inhalation experiments. One hundred and twenty-two rabbits were exposed to atmospheres containing varying volume percentages of nickel carbonyl vapour in air for varying periods. After a few preliminary experiments, the time factor was kept constant in a long series and the concentration was varied. When inhaled for 65 minutes, calculated from the time of establishing a connection between the gasholder and the poison chamber, 0·018 to 0·0188 volume per cent. of nickel carbonyl in air killed 64 out of 77 rabbits, i.e. 83·11 %. It would thus appear that, after making the 14½ minutes allowance as suggested above, 0·018 volume per cent. was capable of killing when inhaled for 50½ minutes. Variations of the time and concentration factors led to the following results :

TABLE I.

	Concentration of Ni(CO)	Ratio of concentration to 1st series	Length of whole experiment	Time after deduction of 14½ minutes	Result
1.	0·018 vol. %	100 : 100	65	50·5	†
2.	0·04 „	222 : 100	40	25·5	R
3.	0·075 „	416 : 100	31	16·5	R
4.	0·016 „	88 : 100	72	57·5	†
*5.	0·015 „	83 : 100	108	93·5	†
6.	0·0136 „	72 : 100	79	64·5	R
7.	0·0134 „	74 : 100	82	67·5	†

* This was one single rabbit and the time was unnecessarily long.

† signifies died. R signifies recovered.

From these figures it will be seen that the concentration of vapour necessary to kill an animal was in inverse proportion to the time the vapour was inhaled.

Cats withstand the effect of the vapour better than rabbits. After inhaling the carbonyl for 90 minutes (i.e. 75·5 minutes after making the deduction) 25 out of 30 animals, i.e. 83·3 %, died when 0·04 volume per cent. of the vapour in the air was used.

Dogs die after inhaling air containing 0.036 volume per cent. of nickel carbonyl for 90 minutes (i.e. 75.5 minutes after the deduction is made).

(iii) *Course of poisoning.*

The effect of the inhalation of the vapour of nickel carbonyl by rabbits may be illustrated by the following account of a typical case.

Rabbit 12 (second series). Weight 1935 grams. Temp. 39.4° C.

0.2 c.cm. of liquid nickel carbonyl was evaporated into the gasholder and to the total quantity of air made up to 192.75 litres, i.e. 0.0188 volume per cent.

The vapour mixture was allowed to pass through the chamber at 10.20 a.m. The respiratory rate was increased after 10 minutes to 132 in the minute, probably on account of apprehension, but soon fell again to 90. After 25 minutes, some uneasy movements were performed. After 34 minutes, the respiration was 176 and the rabbit became extremely restless. The respiration increased in rate steadily from this time and dyspnoea was seen. The restlessness passed off. At the end of one hour, the respiratory rate was 180, the rabbit passed urine and faeces but remained quiet. The visible vessels in the ears did not show any change of colour, but the lips and tongue were cyanotic.

The rabbit was taken out at 11.25, i.e. after having been in the chamber for 65 minutes.

At 11.30, the respiratory rate was 180, the dyspnoea had disappeared and the animal appeared comfortable. The temperature taken at 2.45 p.m. was 38.8° C. and the respiratory rate 120. During the rest of the day the rabbit seemed to be but little affected.

On the following morning the weight had decreased to 1712 grams, the temperature was 38.0° C., the respiratory rate was 196; there was dyspnoea, slight cyanosis of the lips and tongue, and on ausculting the chest, a prolongation, roughening and loudening of the inspiration was heard over the inter-scapular space and also to a less degree in the axillae. This gave the impression of reversed bronchial breathing. The expiratory sounds were unaltered and no râles were heard. The rabbit was taking very little food, but on being allowed out of its cage, was quite lively. During the afternoon, the dyspnoea increased and on the following morning (i.e. 48 hours after the inhalation) it seemed very ill. The weight was 1673 grams, the temperature 38.3° C., and the respiratory rate 204. There was much dyspnoea and cyanosis. It refused food altogether. The physical signs had increased considerably. 24 hours later a distinct improvement was seen, the respiratory rate was 156 and the dyspnoea was not so marked, while the temperature was 39.4° C. The weight however was 1607 grams. At the end of 96 hours, the weight had dropped to 1570 grams, i.e. a loss of nearly 19% of its original weight. The respiratory rate was 132, there was urgent dyspnoea again and extreme cyanosis. The temperature had fallen to 34.5° C., and the animal was apathetic and ill. The physical signs during the last 24 hours revealed consolidation of both lungs, but there was no absolute dulness to percussion. The character of the breath sounds

was more tubular than at first, and there were a few râles to be heard. It died at 1 p.m. i.e. 98½ hours after poisoning.

The following chart shows the variations of temperature and respiratory rate.

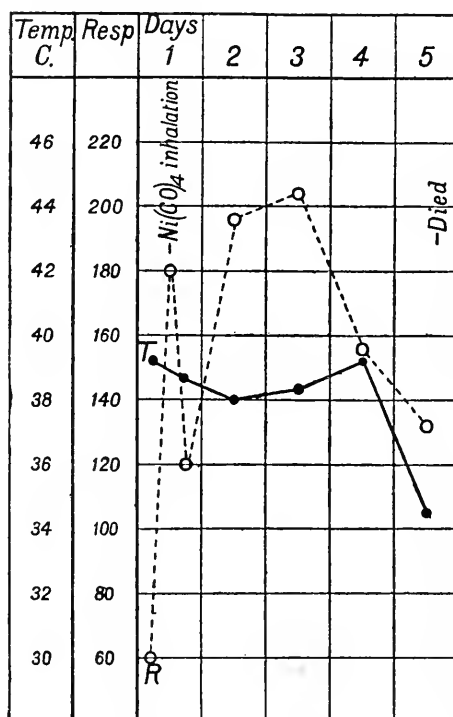


Fig. 2. Chart of Rabbit 12.

(iv) *Variations.*

In other cases, some variations of the symptoms were noted. In cases in which the death took place late, the respiratory rate became very slow but the dyspnoea increased up to the end. In one instance a rabbit died after 204 hours. During the last 12 hours of life, the respiratory rate was only 24 in the minute. When the inhalation was prolonged for a considerable time, and when strong concentrations of vapour were used, the lung symptoms became more intense.

The heart was rarely affected, the rate however usually failing to keep pace with the respiratory rate. Reduplication of the first sound at the apex was heard at times. The peripheral vessels were frequently contracted from the third day onward.

As a rule, symptoms referable to disturbance of the central nervous system were not well marked. It is difficult to decide whether the dyspnoea and cyanosis were entirely due to the changes in the lungs. In a few cases, there was paresis of the hind legs. The muscular tone

was diminished or lost, the muscle most frequently affected being the sphincter ani. From the second day onwards there was loss of sexual desire. Urine was retained for some hours before death in a number of cases. Final convulsions were frequently seen. The animals nearly always assumed an orthotonic position before death.

The temperature curve was of the type shown above in the majority of the rabbits. Modifications however were seen, including the following:

- (a) gradual fall after 24 to 36 hours,
- (b) sudden fall at 24 hours to 35° C. or lower,
- (c) fall within 18 hours, with subsequent rise and later fall.

Death took place between 60 and 90 hours after poisoning in a large number of experiments. The average for the 64 rabbits poisoned by inhaling 0.018 volume per cent. of nickel carbonyl for $50\frac{1}{2}$ minutes was 69.2 hours.

The variations noted included:

- (a) Immediately after poisoning (1 in 122).
- (b) Between 23 and 36 hours (11 in 122).
- (c) Between 100 and 150 hours (4 in 122).
- (d) Over 200 hours (2 in 122).

Some rabbits appeared to be very resistant toward the effect of the vapour and this resistance appears to vary at different times in the same animal. A previous non-fatal poisoning dose appears to render the rabbit slightly less susceptible to the vapour, provided that sufficient time is allowed to elapse for the obvious lesions to be repaired. Independent diseases, such as tuberculosis, render rabbits more susceptible to nickel carbonyl.

In Part I of this paper, reference was made to the examination of the blood for carbon monoxide. The maximum amount of carbon monoxide found in any one case was equivalent to a saturation of not more than 5% of the haemoglobin.

A number of rabbits were poisoned with quantities of nickel carbonyl just sufficient to produce death, and the blood was examined daily at the same time. It was found necessary first to control the blood counts for several days, prior to the poisoning, since some rabbits exhibit considerable variations. Only those rabbits which gave steady counts were used. The number of red corpuscles remained unaltered until the 4th or 5th day, when a reduction to about $\frac{2}{3}$ of the original number was observed. The haemoglobin value fell at about the same time, but the lowest values were only about 85% of the full values.

During the first three days, the leucocytes tended to decrease in number but never reached below the lower limit of a normal count. The slight drop was followed by a recovery to the former level. Just before death a distinct leucocytosis was seen. The polymorphonuclear elements were the only leucocytes increased in number. A curve is appended to illustrate the type of the changes (Fig. 3).

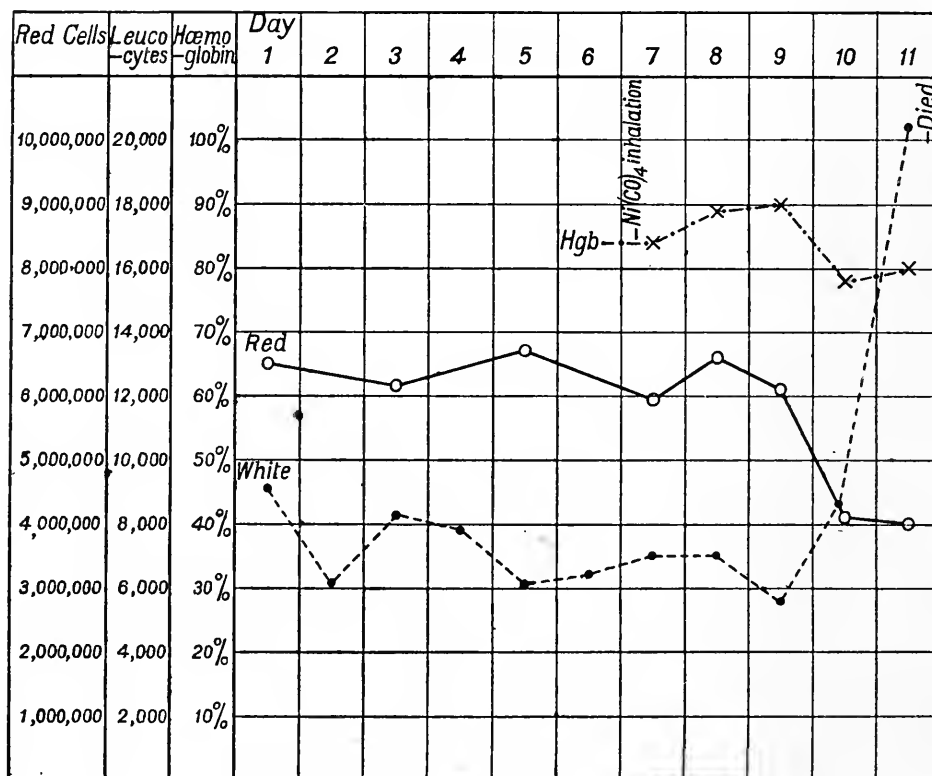


Fig. 3.

(v) *Poisoning in cats.*

The following is an example of the typical course of nickel carbonyl poisoning in cats :

Cat 11. Weight 3340 grams.

0.42 c.cm. of liquid nickel carbonyl was evaporated into the gas holder and the total quantity of air was made up to 189.8 litres, i.e. 0.04 volume per cent.

During the inhalation there was preliminary increase of the respiratory rate, followed by ordinary breathing, and after about 25 minutes, rapid and difficult breathing was evidenced. Toward the end of the inhalation, faeces were passed and the cat vomited. There was salivation. On being taken out at the end of 90 minutes, it was obviously very giddy, dyspnoeic and somewhat cyanotic. At 4 p.m. the temperature was 39.4° C., the respiration was 42 in the minute and the heart was beating 180 in the minute. It was still distinctly affected from the

inhalation but the dyspnoea was markedly less than it was when the cat was taken out of the poisoning chamber. On the following day the weight was 3170 grams, the temperature 38.5°C ., the respiration 66 and the heart beat 156. There was some dyspnoea and the cat was dull and apathetic. The inspiratory sounds were harsh and loud all over the chest, but there were no added sounds, and no impairment of resonance. The dyspnoea increased during the day, and on the following morning was very marked. The weight had then dropped to 3118 grams, the temperature was 36.8°C ., the respiratory rate 114 and the heart beat 102. The physical signs all over both lungs were typical. On the next day the weight still further decreased to 3010 grams, the temperature was 35.2°C ., the respiration 78 and the heart beat 132. The reflexes were distinctly increased, the pupils were dilated but reacted to light, and there was marked cyanosis. Over the right lung, behind, there was impairment of resonance and the expiratory murmur was rough and prolonged in this situation. Otherwise the signs were unaltered. During the following days the weight decreased about 100 grams a day, while the respiratory rate decreased to 54, 30, 24, 22 and 23 on the successive days. The heart rate remained about 144 during the latter part of the illness. The temperature rose on the 5th day to 37°C ., fell slightly on the 6th but rose again on the 7th day to 37.4°C . After this it fell to 33°C . and 31°C . before death. A curve showing the respiration, pulse rate and temperature is appended.

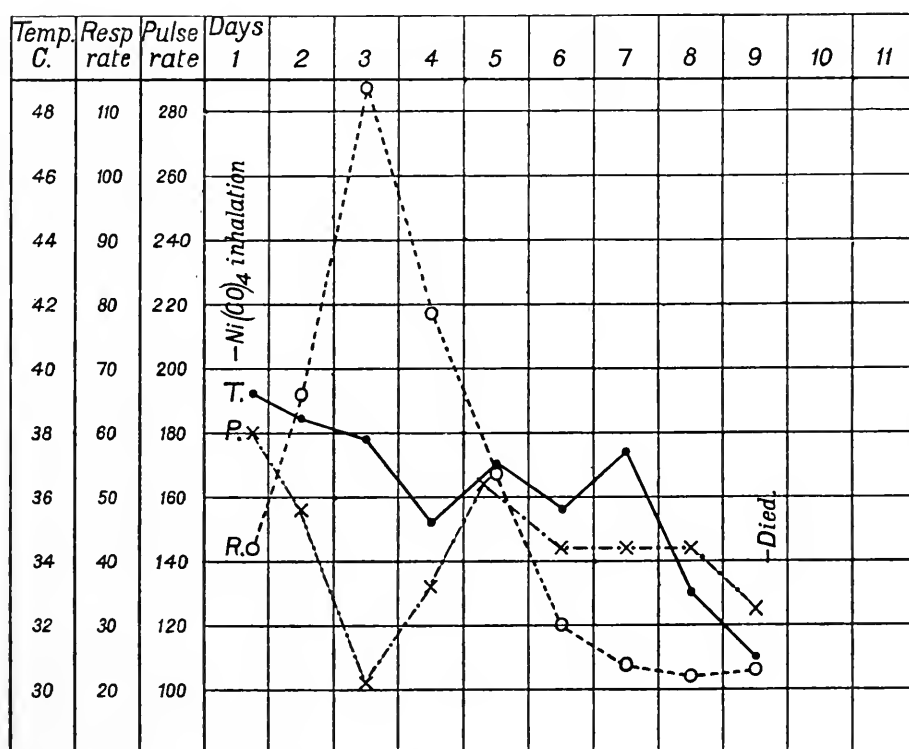


Fig. 4. Chart of Cat 11.

On the 6th day the reflexes became less brisk, and on the 7th day there was distinct paresis with some ataxy of the gait. The signs of consolidation did not increase and on the 7th day a considerable increase in the râles was noted. The

breath sounds became faint. The cat died after about 200 hours, during the night of the ninth day. The loss of weight represented over a quarter of the original weight. There was diarrhoea during the last three days.

(vi) *Variations of symptoms in cats.*

Vomiting during the inhalation was frequently seen. In some cases there was little increase in the respiratory rate during the greater part of this period.

The respiratory rate in the subsequent stages varied considerably. The following gives the range of the variations :

Up to 6 hours after poisoning	26 to 30	per minute.
7 to 24 hours „ „	30 to 48	„ (or more)
25 to 48 „ „	60 to 120	„
49 to 60 „ „	70 to 100	„
61 to 84 „ „	70 to 120	„
85 to 96 „ „	70 to 100	„
97 to 120 „ „	60 to 80	„

The decrease in the rate was marked in some cases, especially when death occurred late. When recovery took place the fall to normal was gradual. During the acute period, laryngeal stridor and a hoarse miaow were often observed.

Death occurred on an average in 88·93 hours in cats which were subjected to the inhalation of 0·04 volume per cent. for 75·5 minutes.

In 1 case, it was delayed to 348 hours.

In 3 cases, it took place between 200 and 250 hours.

In 6 cases, it took place between 100 and 200 hours.

In 4 cases, it took place between 27 and 40 hours and in one case it took place during the inhalation.

(vii) *Poisoning in dogs, guinea-pigs, etc.*

Dogs behave like cats ; they are slightly more susceptible to the poison than the latter. Diarrhoea was present in all the cases. In other respects no differences were noted.

Guinea-pigs proved to be very susceptible to nickel carbonyl vapour. They showed most of the signs and symptoms which are seen in rabbits.

Insects die rapidly in dilute mixtures of the vapour.

A series of experiments was conducted on frog's heart and muscle nerve preparations. The carbonyl was applied in vapour and also in liquid form. No direct effect could be noted. It is however uncertain whether the nickel carbonyl ever reached the muscle cells or nerve fibres, owing to the fact that it is readily dissociated (see Part I) and also to the unfavourable conditions for absorption.

III. PATHOLOGICAL CHANGES.

(i) *Macroscopical.*

As has been stated, rabbits usually assumed a position of orthotonos before death occurred and the cadaver retained this position. In cats, this characteristic was not noted.

Rigor mortis took place normally. No typical external appearances were present after death.

No changes have been seen in the heart after poisoning with nickel carbonyl. In one case, emphysema pericardii was present.

The Lungs.

The changes met with in the lungs consisted of congestion, oedema, haemorrhage and compensatory emphysema.

When death occurred during the first 24 hours, the hyperaemia was often the only change present. In the majority of cases, however, there was fairly well marked oedema and at times a few punctate haemorrhages were seen on the pleural surface and on section. Parenchymatous haemorrhages of considerable size were rare at this stage.

When death occurred during the second day, the hyperaemia was intense. The lungs were freely bathed in a blood-stained fluid. Numerous small and medium sized haemorrhages, not only on the pleural surface, but also pervading the whole organ were common. Large haemorrhages were less frequent. In some cats, patches of consolidation were seen at this stage.

When death took place during the third day, small patches of more or less complete consolidation were usually present. These patches were of dark mulberry colour and exuded blood when squeezed. The haemorrhages which affected considerable areas of lung also gave the appearance of consolidation. They were large or small and were usually scattered over the whole organ, save in those situations in

which emphysema existed. The emphysema was best seen at the free edges of the lobes and a characteristic mosaic-like appearance was seen on the pleural surface. Oedema was rarely clearly seen by the naked eye at this stage on account of the other changes which obscured it.

When death took place during the 4th or 5th day, the infiltration with blood-stained fluid, the consolidation and haemorrhagic foci had reached their maximum. A few fresh haemorrhages were found in a few cases but the majority were of older standing. Whole lobes or even whole organs presented an almost uniform mulberry colour and had the consistence of liver. The tissue sank in water. On section, a frothy blood-stained fluid could be expressed.

Pleural effusion was found in one case.

The Bronchial Glands.

In cats, the bronchial glands were seen to undergo changes in keeping with those in the lungs. At first there were some swelling and redness. The colour then became darker and small haemorrhages were seen. Dark specks were seen in the substance after about 36 hours; these specks sometimes had a greenish tinge. Later, the haemorrhages became more intense.

Trachea, Larynx and Bronchi.

In practically all the cases, the mucous membrane of the upper air passages was markedly injected for the first few hours after the inhalation. Haemorrhages were sometimes seen early, but more often they occurred at a later stage. The passages often contained a viscid greenish fluid or else a clear frothy or blood-stained fluid.

Liver, Pancreas, Spleen, Kidneys, Bladder and Genital Organs.

Changes in these organs were seen only on rare occasions. In a few cases, haemorrhages were met with in the kidneys, spleen, pancreas, ovaries, testes and uterus.

Intestines and Stomach.

Haemorrhages were seen at times in the mucous membrane of the stomach and very rarely in that of the intestines. Congestion and ulceration were also met with exceptionally.

Adrenals.

Changes in the adrenals were met with in the majority of the cases. These were first seen after the 2nd day. Small localized or large diffuse haemorrhages were common and general swelling and redness of the organs usually accompanied them. At times there was only congestion. The haemorrhages were usually situated in the medulla of the adrenals.

Brain.

Changes were commonly met with in the brain and were sometimes extensive. The meninges were generally suffused with blood, the vessels were dilated and at times the membranes thickened. In rare cases, some meningeal haemorrhages were seen. The changes were met with from the 2nd day onward and were the more intense the later death took place.

As a rule, the white matter of the brain was the seat of haemorrhages. These were generally minute. They were seen in the *optic thalami*, *corpus striatum*, internal capsule and *pons varolii*, as well as in other situations. In the medulla and cerebellum, haemorrhages were also seen in a number of cases. Simple congestion was met with frequently. Extensive haemorrhages in the brain of rabbits, cats and dogs, like those observed in the brain of one of the human subjects, who died of plant gas poisoning at the Nickel Works, have not been met with.

The Spinal Cord.

In a few cases, in which the spinal cord was examined, no pathological changes were seen.

The following table shows the frequency of haemorrhages met with in the various organs.

TABLE II.

Showing the frequency of haemorrhages in the various organs.

	Cats (31)	Rabbits (93)
	100 %	100 %
Lungs	100	100
Liver	6.4	4.3
Spleen	0	2.1
Pancreas	3.2	1.0
Kidneys	0	6.4
Adrenals	83.8	51.6
Stomach	0	9.6
Genital organs	0	5.3
Brain	74.1	37.6

(ii) *Microscopical.*

It is only necessary to describe the microscopical appearances of the lungs, bronchial glands, adrenals and brain, as the changes met with in other organs were rare and corresponded exactly to the macroscopical appearances.

The organs were fixed and hardened in potassium bichromate and acetic acid or Flemming's osmic acid fluid followed by alcohol. Brains were either put through alcohol, bichromate of potassium, formaline or Muller's solution or else fixed by Marchi's method.

The Lungs.

Oedema is the most striking of the early changes. The specimens show a diffuse homogeneous material, which stains more or less deeply with eosin. The fluid is usually situated in the pulmonary tissue, lying like a stroma in the network of the interalveolar tissue. At times, however, it pervades the spaces and may be seen either in the alveoli or even in the bronchioli and bronchi (see Plate VII, Figs. 1 and 2). Often the fluid contains a few blood corpuscles.

Haemorrhages vary considerably in extent. They may be so extensive that the lung structure is quite obliterated. The blood cells are first poured out into the interalveolar tissue and pass into the air vesicles and passages later. In early cases, diapedesis may be marked. The endothelial cells of the capillaries at first appear swollen, then degenerative changes are seen, then the wall gives way at one spot and the blood issues out, gradually enlarging the defect produced in the wall.

Leucocytosis in the vicinity of the capillaries is often extensive in limited areas, and after a time, peribronchitic small cell infiltration is seen. The alveoli and smallest bronchioli become more or less filled with blood cells and the appearance when whole lobules are affected only differs from that of typical broncho-pneumonia by the occasional presence of the homogeneous fluid and haemorrhagic patches. In advanced cases, there is a typical inflammatory process, identical to that of pneumonia, including the polynuclear exudate in the alveoli, pus cells in the bronchi and well marked desquamation of the endothelium. At times, one meets with some proliferation of the bronchial epithelium. In the earlier stages, the polymorphonuclear cells are usually absent and the infiltration is then limited to small round cells.

The Bronchial Glands.

Considerable congestion, small cell infiltration and haemorrhages may be seen quite early. The lymphoid tissue appears at first to be but little affected, since the leucocytosis and haemorrhages are not extensive. Later on more extensive haemorrhages may occur. In some situations minute intracellularly placed granules of dark colour may be seen. At times, the endothelial cells take up red blood corpuscles.

The Adrenals.

The changes met with correspond to those in the lungs. In the earliest stages, the endothelial cells of the capillaries undergo degeneration, then they rupture and the blood cells are poured out into the surrounding tissue. Later on the parenchymatous cells may degenerate secondarily (see Plate VIII, Fig. 5). During the early stages, leucocytosis may be seen in the neighbourhood of the capillaries. Primary changes in the parenchymatous cells cannot be detected, but the fact that haemorrhages frequently obscure the early changes must be taken into account before one can definitely exclude the possibility of their existence.

The Brain.

In this organ, the changes are like those met with in the lungs. The earliest changes seen are found in the walls of the capillaries and small blood vessels. The endothelial cells are swollen, the nuclei are badly stained and the outlines of the cells are not well differentiated. Many of the cells are misshapen. When fixed by osmic acid, fat globules are seen both below the endothelial layer and in the endothelial cells themselves (see Plate VIII, Fig. 6). In a few situations, fat is seen in the lumen of the vessel, in cells lying free. These cells appear to be like endothelial cells and it is probable that they had been cast off.

After the cells have become sufficiently affected, they are no longer capable of withstanding the pressure of the blood and thus the vessel gives way, at first by the failure of one or two cells and later by a number of contiguous cells becoming destroyed or pushed aside. The blood escapes from the vessel in comparatively narrow tracks and the cells are found closely packed together (see Plate VIII, Fig. 7). A considerable amount of leucocytosis is seen in the neighbourhood of

the affected capillaries, especially before the rupture takes place. The leucocytes are frequently grouped around nerve ganglion cells and appear to be connected with the changes which take place in the latter, even before a haemorrhage takes place. At first, the Nissl's granules become indistinct and stain weakly. Later on, they become more and more ill defined until they disappear and the contents of the cell appears to be homogeneous (see Plate VIII, Fig. 8). During the later stages, the processes of the ganglion cells can no longer be demonstrated. In the majority of cases, the chromatolysis does not become complete.

Secondary degeneration of the nerve fibres is not often seen in cats and never in rabbits, since the time between the inhalation and death is too short for such processes to be marked. Slight Marchi degeneration was seen in the case of a cat, in which repeated sub-lethal doses of nickel carbonyl were given, with the effect that the cat lived for 22 days, during which time it was continually under the influence of nickel.

IV. ATTEMPT TO TRACE THE NICKEL THROUGH THE ORGANISM.

(i) *Methods of detection.*

The detection of nickel in the various tissues was carried out in two ways. Firstly the organs were ashed and the nickel was determined quantitatively by the dimethyl glyoxime method, described by Harden and myself (1906)¹. Secondly the nickel was recognized by micro-chemical means. The first method was necessary in order to obtain information with regard to the passage of the nickel and the rate of its passage, while the second method was found only to be available during the earliest stages in the lungs, before the nickel had entered into complex combination.

Considerable difficulty was experienced in working out a satisfactory method of micro-chemical detection of nickel in the tissues. Two reactions were found to be applicable. The first of these is the dimethyl glyoxime reaction, but this has a great disadvantage in that the compound first forms in solution and subsequently crystallizes out. The appearance of the sections, however, was very characteristic, when the preparation was carried out as follows. A rabbit was exposed to a high concentration of nickel carbonyl vapour and on being removed

¹ Harden and Armit. *Proc. Roy. Soc.*, 1906.

from the poison chamber was at once killed. The lungs were removed and portions were cut fresh by the freezing microtome. The sections were first floated out on a weak solution of sodium triphosphate, to prevent a solution of the nickel, then they were transferred to a solution of dimethyl glyoxime in methyl alcohol, to which a few drops of ammonia had been added and allowed to remain in this solution for several minutes. They were then rapidly passed through water containing a few drops of ammonia and spread out on slides. The specimens were examined either unstained or faintly stained in methylene blue. The nickel appeared as a diffuse red staining of the tissue, which however paled rapidly and from which red needle shaped crystals separated out. The position of these crystals was no indication of the position of the nickel. The other method showed that the temporary appearances observed by means of the first method actually corresponds to the position of the nickel.

Another method for the detection of nickel in the tissues was employed. A rabbit was poisoned by inhalation of nickel carbonyl in air for 25 minutes. After an hour during which the nickel carbonyl would be entirely dissociated, it was killed. The lungs were removed and blown up several times with sulphuretted hydrogen for about thirty minutes. They were then blown up with an indifferent gas (carbon dioxide was used), which first passed through a tube immersed in boiling water. The lungs were kept during this stage in a dry vessel at a temperature of 90° C. After the lungs had been heated in this way for three hours, portions were removed and cut by the freezing microtome. The sections were floated out on water, passed through spirit and again floated out on water. They were then spread out on slides and stained with haematoxylin. The nickel was seen as a brownish staining of the tissues. On examining the affected parts with a high power, very minute granules of a brownish colour could be detected. A similar appearance was seen when a solution of the dissociation product of nickel carbonyl in serum (see Part I of this paper) was treated with sulphuretted hydrogen and the serum coagulated by heat was examined under the microscope. The solution of the nickel sulphide in serum is a dark brown almost clear solution, but when seen under a high magnification, it is found to contain minute granules of dark colour.

(ii) *Evidence of what takes place in the lungs.*

Fate of nickel carbonyl when inhaled in the lungs.

In tracing the nickel carbonyl through the organism, it is necessary to enquire what happens at the site of absorption. It is therefore desirable to consider the immediate result both to the carbonyl and to the lungs when the vapour is inhaled. It has already been shown in Part I of this paper, that nickel carbonyl vapour when placed in contact with air and moisture at the temperature of the animal body dissociates rapidly. Evidence that dissociation takes place in the air passages is found in the deposition of a nickel containing substance on the surface of the mucous membranes. In order to follow the process, it is necessary to consider what amount of nickel is available for absorption and also in what way and with what rapidity it is absorbed by the blood.

Direct measurement of the quantity of nickel carbonyl dissociated could not be carried out, on account of the difficulty presented to accurate analysis by the small differences in the volume per centage of the already dilute gas mixture before and after passing through the poison chamber.

Indirect evidence is however obtainable. The maximum amount of nickel carbonyl available is calculated by determining the amount of air mixture which the animal breathes during the experiment. As has been stated, the inhalation of 0.018 volume per cent. of nickel carbonyl vapour in air for 50.5 minutes suffices to kill rabbits. A rabbit weighing 2 kilograms takes in about 600 c.cm. of air per minute under normal conditions. It would therefore breathe about 32 litres during the inhalation. The total amount of nickel carbonyl vapour breathed would therefore be about 5.9 c.cm or 0.045 gram. This would contain about 0.0155 gram of nickel. The total quantity of nickel carbonyl entering the lungs would correspond to about $7\frac{1}{2}$ mgrs. of nickel per kilogram body weight.

The animal would not absorb all the vapour which is contained in the air entering the respiratory passages, and evidence that some of the vapour is not absorbed is found in the fact that the expired air of an animal immediately after the inhalation contains recognizable quantities of nickel carbonyl. The amount mentioned above must therefore be regarded as a store from which the animal will abstract a fraction, which will serve as a lethal dose.

In cats, the inhalation of 0.04 volume per cent. of nickel carbonyl in air for 75.5 minutes proved sufficient to kill. A cat weighing 3 kilograms breathes about 800 c.cm. per minute. This would correspond to about $60\frac{1}{2}$ litres during the inhalation. This is equivalent to about 24.3 c.cm. or 0.184 gram of nickel carbonyl or roughly 64 mgrs. of nickel. The available amount of nickel carbonyl expressed as nickel would therefore not exceed 21 mgrs. per kilogram body weight.

In the case of a cat which was affected with an old pulmonary lesion, probably of tubercular origin, and which died at the end of the inhalation before it could be removed from the poison chamber, 32 mgrs. of nickel were recovered from the lungs after ashing, while the blood was found to be free from nickel. The cat weighed 3800 grams, so that 8.4 mgrs. of nickel per kilogram body weight was found. In other cases, the amount recovered from the lungs was less than this amount, since absorption from the lungs takes place early. The average amount recovered was about 10 mgrs. The amount found in the blood of cats immediately after inhalation varied up to 3.75 mgrs. per 100 grams of blood. Thus in cats weighing up to 3 kilograms, from 20 to 22 mgrs. of nickel has been accounted for in the lungs and blood, while small quantities would probably be present in other tissues (e.g. the bronchial glands usually contain small quantities early). Making some allowance for this and having regard to the result of the experiment in which 32 mgrs. was recovered, it may be assumed that after inhaling 0.04 volume per cent. of nickel carbonyl vapour in air for $75\frac{1}{2}$ minutes, cats are able to dissociate an amount of nickel carbonyl representing not less than $8\frac{1}{2}$ mgrs. of nickel per kilogram body weight. This would correspond to between $\frac{1}{3}$ and $\frac{1}{2}$ of the total available amount. In rabbits, up to $3\frac{1}{2}$ mgrs. of nickel per kilogram body weight has been accounted for in the same way immediately after inhalation. The absorption of about one half of the inspired quantity of a toxic gas has been met with in the case of carbon monoxide (Haldane, 1895).

It has already been stated that from the earliest stage of nickel carbonyl poisoning, the lungs undergo changes. It is uncertain whether the vapour as such acts as an irritant. Nickel carbonyl is dissociated into carbon monoxide and a nickel containing product which is probably a hydrated sub-carbonate of nickel (see Part I, p. 541 et seq.). The quantity of the carbon monoxide is too small to produce any appreciable effect, since the amount of carbon monoxide liberated from the nickel carbonyl corresponding to $8\frac{1}{2}$ mgrs. of nickel would be less than $9\frac{1}{2}$ c.cm., and this small amount would be available

for absorption during the $75\frac{1}{2}$ minutes (see also Part I, p. 528). The nickel containing substance is gradually dissolved by the tissue fluids and the dissolved nickel acts locally upon the peribronchial and alveolar tissue as well as on the endothelial cells of the capillaries producing the changes which have already been described. The production of a serous effusion or oedema hastens the solution of the deposited nickel compound. At first, the nickel containing product of dissociation is simply dissolved, without any alteration of its chemical constitution, but after the course of some hours, it becomes acted on by the proteid material and a more complex nickel combination results. The evidence of this is found in the fact that at first the red compound forms with dimethyl glyoxime readily, but later on this combination only takes place slowly, and after about 24 to 36 hours although there is still nickel in the lungs, as demonstrated by analysis after ashing, neither the dimethyl glyoxime compound nor the sulphide can be formed.

At times, the absorption takes place very rapidly and in one case of a small rabbit, less than 1 mgr. of nickel was found in the lungs immediately after poisoning. The process of absorption however takes some time to complete and 3 mgrs. has been found 24 hours and 1 mgr. 48 hours after the inhalation in rabbits. From this time onwards only small traces could be detected and although 0.035 mgr. was found in one rabbit after 96 hours, the nickel was usually completely removed after about $2\frac{1}{2}$ days.

The microscopical appearances assist in determining the way in which the nickel is taken up. The free surfaces and the tissue immediately surrounding the surface of the bronchi, bronchioli and alveoli show red or brownish staining when the sections have been treated by the dimethyl glyoxime or sulphide methods. The study of the dissociation of nickel carbonyl described in Part I has shown that under the conditions of temperature, moisture and carbon dioxide obtaining in the lungs, the dissociation product is formed, which is slightly soluble in water and in solutions of phosphates. It is thus clear that the nickel subcarbonate is deposited on the respiratory mucous surfaces and is gradually dissolved from this situation by the tissue fluids. This accounts for the intense staining at the free edges and the diffuse staining in the neighbouring tissues.

(iii) *The absorption of the nickel by the blood.*

Evidence of the absorption of the nickel deposited on the respiratory surface and dissolved from this situation by the tissue fluids may

be obtained from (1) the presence of nickel in the blood during the early stages, (2) the gradual disappearance of the nickel from the lungs, (3) the appearance of nickel in other organs, and (4) the appearances of the micro-chemical tests in the lungs during the early stages.

(a) The fate of nickel carbonyl in artificial perfusion experiments.

In following out the fate of nickel carbonyl after inhalation, a short series of experiments with artificial perfusion of the lungs was carried out. It was found that when cats' lungs were employed, the experiments were unsatisfactory, as the tissues became oedematous early and thus terminated the experiment before sufficient time had elapsed. Dogs' lungs were therefore used.

The experiments were carried out with an apparatus devised by Martin and Embley (1905)¹. The lungs were removed and the respiration was maintained by inflating them rhythmically with air containing varying amounts of nickel carbonyl vapour. The animal's own blood defibrinated and kept at 37° C. was used, which was circulated through the lungs. Samples of blood were taken from time to time, and ashed to determine the quantity of nickel which had been taken up. At the end of the experiments, the lungs were also ashed and examined quantitatively for nickel.

The results of two experiments conducted in this way were as follows:

TABLE III.

	Duration in mins.	Amount of air used (litres)	Amount of Ni (CO) ₄ used (grams)	Vol. % of Ni (CO) ₄ vapour	Blood (grams)	Mgrs. Ni per 100 grams blood	Mgrs. Ni in lungs
1.	90	891	0.9436	0.014	300	(a) 0.05 (b) 0.019 (c) 0.037	3.00
2.	50	450	8.8188	0.251	250	(a) 1.75 (b) 1.91	25.87

From these figures it would appear that although the greater part of the nickel is left in the lungs, some of it is taken up by the blood. The amount in the blood is larger the more concentrated the nickel carbonyl vapour. In the first experiment about 0.1 mgr. of nickel appeared in the total quantity of blood used and in the second

¹ Martin and Embley. "The action of anaesthetic quantities of chloroform upon the blood vessels of the bowel and kidney; with an account of an artificial circulation apparatus." *Journ. Phys.* xxxii. p. 147. 1905.

experiment about 4.5 mgrs. These quantities are extremely small as compared with the 3 mgrs. and 25.8 mgrs. found respectively in the lungs.

The first sample taken in the second experiment was found to contain carbon monoxide corresponding to 21.4 % of the quantity necessary to saturate. This is quite consistent with what has already been said on the subject of carbon monoxide absorption (see Part I, p. 529), since the concentration of the nickel carbonyl vapour was considerably greater than was employed in the inhalation experiments on animals.

The conditions of these experiments are not exactly similar to those of inhalation by a living animal. The surface of the lung was considerably cooler and the tissues were in a dying condition. The experiments, however, show that dissociation of nickel carbonyl takes place rapidly in the lungs at body temperature and that the nickel is at first deposited in the lungs to be gradually dissolved and carried away by the blood. In the experiments it is possible that some nickel carbonyl went into solution in the blood but it would be rapidly dissociated before it had time to leave the blood again. The fact that the lungs became oedematous and thus terminated the experiments when high concentrations were used sooner than when lower concentrations were used points to a direct toxic action of the nickel on the tissue.

(b) Rate of absorption of nickel from the lung.

The blood, as has already been shown, contains varying quantities of nickel during the early stages. At times no nickel was recovered after ashing small samples within the first day, but as a rule, especially when 50 grams were ashed, easily detectable amounts were regained. The rate of the removal of nickel from the pulmonary tissue will depend on the conditions of the circulation obtaining for the time. The presence of a finely divided nickel compound on the mucous surface produces an inflammatory reaction. The leucocytes and inflammatory cells increase the density of the organs. A serous exudation is thrown out and the air passages become more and more encroached upon, and as a result parts of the respiratory surface become obliterated. It has further been shown that nickel coming into contact with the capillary walls causes changes of a fatty degeneration (this was best demonstrated in the vessels of the brain). The cells give way and one of the results of the haemorrhages which ensue is that the organs become still denser. The circulation through the lungs is thus slowed down by the resistance of the solid organ and by the mechanical interference to the circulation brought about by the rupture of the capillaries and consequent coagulation. Owing to the slowing of the blood stream, the nickel finds it

increasingly difficult to get out, and as a result nickel can still be found in the lungs at the end of 60 hours or at times even later.

The quantity of nickel during the second and third day which is present in the blood at any given time must be extremely small, and it is therefore not surprising that none was detected during the second day in any of the analyses. As will be mentioned later, there is reason to believe that nickel is rapidly claimed by other tissues, and this would therefore lessen the quantity present in the blood at any given time. Little by little however the nickel is washed out of the lungs into the blood stream. A certain amount finds its way into the bronchial glands through the lymphatic channels. It can therefore be said that the amount taken up by the blood and available for other tissues at any given time is inversely proportionate to the acuteness of the onset of the pneumonic processes. The more slowly the consolidation of the lungs takes place, the larger will be the amount of nickel which enters the circulation at any one time.

(iv) *Deposition of nickel in the tissues and organs.*

It has been stated that evidence of the absorption of the nickel was obtained by the appearance of nickel in other organs. The various organs and tissues were analysed for nickel at the various stages of the poisoning, but no nickel was detected at any time in the liver, spleen, pancreas, muscles or bile. A very small trace was found on one occasion in the heart immediately after the inhalation, but this was probably due to the accidental inclusion of some blood clot. Nickel was found in the bronchial glands within a short time after poisoning. In the kidneys, nickel was only found at a late stage. This fact will be referred to when the subject of the elimination of the nickel is discussed. The adrenals contained nickel from the 36th hour after inhalation onward. As a rule, the quantity was very small and at the later stages, it did not exceed 0.02 or 0.03 mgr. It was also found in the brain regularly after the end of the first day until the end of the fourth day. A table is appended which shows the position of the nickel during the successive stages of the poisoning.

Evidence of selective absorption by certain tissues.

The results of these analyses point to a selection on the part of the adrenals and brain. The nickel must be available for all the organs, but save for an occasional lesion in other organs, there is no evidence of the presence of the toxic metal in them. The blood yields up its nickel

to the adrenals, and brain during the course of the second, third and fourth days. A certain amount of nickel is found in the bronchial glands early, but this is more probably due to the absorption of the nickel either in solution or even in solid form in the bodies of leucocytes through the lymphatic channels. The presence of nickel in the kidneys and the occurrence of pathological lesions in these organs may be neglected for the time being. It must also be pointed out that the lesions

TABLE IV.

Showing the localisation of the nickel in the tissues and organs at varying times.

	0—12 hrs.	12—24 hrs.	24—36 hrs.	36—48 hrs.	48—60 hrs.	60—70 hrs.	Later
Lungs	+++	+++	++	++	+	0	0
Bronchial glands	+	++	++	++	...	0	...
Stomach	0	0	0
Adrenals	...	0	0	++	++	++	+
Brain	0	×	×	++	+++	++	+
Kidneys	0	...	0	...	+	++	+
Blood	++	+	0	0	++	++	+
Milk	++
Faeces	0	...	++	++	++
Urine	0	0	+	++	++	+++	++
Bronchial secretion	++	++	++

+ indicates present at times. ++ indicates present.

+++ indicates present in comparatively large quantities.

0 indicates absent. × indicates present on rare occasions.

found in the organs are practically limited to the lungs, adrenals and brain, both in nickel carbonyl poisoning and also, as will be shown later, in poisoning by nickel and its other compounds. The urine contained nickel from the 24th hour till the fourth day or later, and this would further support the contention that the nickel does not remain long in the organs but passes through them, producing damage during a short stay. One is therefore unable to present evidence typical of selective absorption, namely a definite proportion between the quantity of the nickel and the mass of the organ. The maximum amount of nickel found in the brain was about 0.01 % of the weight of the organ and 0.006 % of the weight of the organ in the adrenals. In spite of this, however, the failure of detecting any nickel in the liver and other organs and the localisation of the pathological lesions must be regarded as evidence of selection on the part of the adrenals and brain.

(v) *Elimination.*

Nickel is excreted in the urine and faeces. On one occasion the milk of a lactating cat was examined and was found to contain nickel on the third day. No nickel was found in the stomach contents, save once in the case of a rabbit in which death occurred early. It is however probable that some bronchial secretion had been swallowed, as this contained nickel.

About 75% of the nickel excreted is found in the urine in rabbits, while in cats over one half of the total quantity is got rid of in this way. The rate of elimination through the kidneys was found to be very irregular, the maximum however being usually reached on the fourth to the fifth day. When the urine contained comparatively large quantities of nickel, some metal was also found in the kidneys. The changes found in these organs were probably produced at this stage, since no congestion or haemorrhage was met with when death took place before the end of 60 hours after the inhalation.

The quantities of nickel in the faeces also reached a maximum about the fourth or fifth day. Diarrhoea usually set in about this period and it was found that the amount of nickel was always greater when the stools were fluid. The faeces then assumed a darkish colour. The nickel cannot be extracted by the action of water or dilute acids and it is probably present in the form of sulphide.

At times death took place after all the nickel had been excreted.

The nickel in the urine appeared to be in a condition of complex combination. Urine containing nickel does not give the dimethyl glyoxime or the sulphide reactions, but after boiling with concentrated nitric acid and neutralising with ammonia both reactions can be obtained. The nickel cannot be precipitated by heat. The urine was subjected to dialysis. After 24 hours, the process was incomplete. In one case, 90 c.cm. of urine was dialysed with 400 c.cm. of water. At the end of 24 hours, 30% had passed through the membrane, while nearly 60% was left inside the skin, the remainder having been absorbed into the membrane. This subject will be again referred to when the excretion of metal in cobalt poisoning is discussed.

An attempt was made to remove a possible organic compound of nickel from the tissues by means of certain solvents (aether, alcohol, saline fluid and water). While aether failed to extract any nickel, the other solvents removed part of the metal. The amounts extracted were very irregular and in no case was the whole amount extracted.

V. POISONING WITH NICKEL AND NICKEL SALTS.

Since nickel carbonyl poisoning is actually poisoning by nickel, in which the metal is applied to the enormous area of the respiratory surface, the symptoms and pathological changes met with in nickel poisonings should correspond with those met with in nickel carbonyl poisoning, allowance being made for the mode of entry.

Laborde and Riche (1888) found that when the soluble salts of nickel are given by mouth, vomiting is produced, but even when 3 grams of nickel sulphate is used, death does not follow. A. Riche (1888) however met with late death after feeding with food impregnated with nickel sulphate in dogs. Death has been seen when the sulphate was injected subcutaneously and intravenously by these two observers. The lethal dose of nickel sulphate applied in this way was found to be 0.0655 gram for rabbits, while 0.378 gram was required for dogs.

Nickel sulphate however is an extremely unsuitable salt for toxicological experiments, as it coagulates albumin and whether injected subcutaneously or intravenously, it is only absorbed very slowly and incompletely owing to the small surface for absorption of the coagulated mass. According to Stuart (1884) the lethal dose of nickel (given as NiO) for rabbits and cats is about 8 mgrs. per kilogram body weight, while for dogs it is higher.

The nickel compounds dealt with in the present investigation are the carbonate, the tri-phosphate, the acid phosphate, the pyro-phosphate, the hydrate, the sulphate, the chloride, the acetate, the ferro-cyanide, the sulphide, the tartrate and the potassium albuminate. Metallic nickel in a state of very fine division was also used. The animals experimented on were guinea-pigs, rabbits and cats. The various preparations were introduced by subcutaneous, intravenous and intra-peritoneal injection.

1. Subcutaneous injection.

0.0023 gram of nickel in the form of sulphate, phosphate, ferro-cyanide and chloride killed guinea-pigs. The same dose of nickel in the form of acetate also killed. This corresponds to about $6\frac{1}{2}$ mgrs. of nickel per kilogram body weight. 0.008 gram of nickel as carbonate was required to kill guinea-pigs. The lethal dose by this method for rabbits with the soluble salts was found to be between 7 and 8 mgrs. per kilogram body weight. Cats died after the injection of 0.23 to 0.03 gram of nickel as nickel sulphate. The lethal dose for cats per kilo-

gram body weight thus appears to be from 9 to 16 mgrs. calculated as nickel.

2. Intravenous injections.

A few experiments were conducted with intravenous injection but the method was discarded when it was found that the soluble salts, such as the sulphate and chloride, and the insoluble salts, such as the hydrate and carbonate, mostly blocked up a small vessel and unless injected in comparatively large quantities or in minimal quantities in several situations, rarely produced symptoms.

3. Intraperitoneal injection.

The insoluble salts were freshly precipitated and injected with aseptic precautions into the peritoneal cavity. Especial attention was paid to the behaviour of the physically indifferent salts, such as the hydrate, carbonate and phosphates. Guinea-pigs died after receiving 5 mgrs. per kilogram body weight, but after death some unabsorbed salt was found in the peritoneum. When larger doses of these salts were used (e.g. 12 mgrs. of nickel) the unabsorbed material could be recovered to a great extent and estimated after dissolving in acetic acid. From these estimations, it would appear that death follows after the absorption of between 3 and 4 mgrs. of nickel per kilogram body weight.

The tartrate was further used to determine the lethal dose, and it was found that rabbits died after the injection of 8 mgrs. of nickel per kilogram body weight. It was noted however that the absorption of the tartrate is more complicated than that of the salts containing free nickel ions, and from the experiments conducted with the acetate, hydrate and phosphate, it appears that the minimum lethal dose for rabbits is less than 7 mgrs. of nickel.

Nickel sulphide was injected into the peritoneal cavity. 0.025 mgr. (calculated as nickel) led to death in two out of three guinea-pigs. After death a large quantity of black material was found in part free and in part adherent to the surface of the peritoneum. Around the adherent particles, a distinct greenish colour was seen at the periphery. The tissue was removed and treated with acetic acid. Nickel was found in the filtrate, showing that the sulphide had been reduced and that the nickel had combined with oxygen or carbon dioxide.

Metallic nickel was prepared from the hydrate or from the oxalate by drying and reducing in hydrogen at about 260° to 280° C. and sealing up the tubes in which the reduction had been carried out. The metal prepared in this way existed in a state of very fine division and was pyrophoric (i.e. took fire spontaneously when exposed to oxygen). Some

of the samples worked with were less finely divided than others. The end of the tube was broken off and a little well boiled physiological saline solution was immediately run into the tube, before the hydrogen in the tube had time to diffuse. The metal was then suspended in the fluid by rapid rotatory movements and sucked up into a glass syringe, without any contact with air. The suspension was injected into the peritoneal cavity of guinea-pigs and rabbits.

The exact dosage was difficult to determine owing to two circumstances. Firstly a little material was always left in the tube, syringe and needle, and secondly after death a varying amount chiefly comprising the coarser particles and clumps which had formed was found unabsorbed. In guinea-pigs, the quantity injected varied between 12 and 28 mgrs. and all the animals thus treated died save a few in which metal which was not very finely divided was injected. 28 mgrs. of nickel in a state of only moderately fine division failed to kill a rabbit, while 30 mgrs. of the same sample killed a second rabbit. Post mortem, relatively large quantities of black material clumped in patches were found in the peritoneum. In every case it was evident that only a part of the nickel injected had been absorbed.

The absorption of the nickel was studied further in these cases. The mesentery which contained small macroscopically visible deposits of black material was stretched on cork discs, fixed, transferred to cover glasses, stained and mounted. In these one could see that the very minutest particles had been taken up by the polynuclear cells and to a less extent by the endothelial cells and by some of the red blood corpuscles. Phagocytosis of the red cell was also seen in situations where the red cells had taken up nickel particles. Nickel granules were further seen in the walls of the lymph spaces, lying free. No granules either intracellularly or extracellularly placed were seen in the blood vessels. The taking up of the nickel appeared to be most extensive about 24 hours after the injection but even as late as 70 hours it could still be seen.

Experiments were further carried out *in vitro*. It was found that pyrophoric nickel, nickel sesquioxide and nickel sulphide in fine state of division are readily taken up by the polymorphonuclear cells.

Symptoms and pathological changes in nickel poisoning.

Guinea-pigs poisoned by nickel and nickel salts all exhibited the same symptoms and post mortem changes. The former consisted in dyspnoea, paresis, at times paralysis and later diarrhoea, cyanosis and convulsions. The lungs showed haemorrhages, compensatory emphysema and some oedema and the adrenals showed haemorrhages.

Rabbits exhibited symptoms like those of nickel carbonyl poisoning, with the exception of the early dyspnoea and giddiness. The respiratory rate became increased after a few hours, the temperature began to fall, the appetite was lost and the animal became dull and listless. These symptoms increased steadily until the dyspnoea became intense and was accompanied by cyanosis. Paresis was only seen in a few cases. Diarrhoea was usually seen after about 24 to 36 hours and death followed in the course of from a few hours to three or four days. The temperature sank to a low level before death.

In cats, the subcutaneous application of soluble salts produced characteristic symptoms. At first general illness without any definite signs was seen, later the appetite was lost, the weight fell and vomiting and diarrhoea set in. Paresis or even paralysis was noted and the gait became ataxic. Tremors of the anterior extremities and of the head occurred. The respiratory rate increased within a short time. Death occurred within the first 12 hours when large doses were injected, while when smaller doses were employed, it was delayed for some days but not exceeding eight days.

Post mortem the lungs, adrenals and brain showed similar changes to those seen after nickel carbonyl poisoning. The pulmonary changes were not so extensive or intense as those met with in nickel carbonyl poisoning, presumably because the whole quantity of the metal does not pass through the lungs as it does when the vapour is inhaled. When death took place late, large areas of lung tissue were affected, and it was impossible to distinguish the sections from those of nickel carbonyl poisoning. The kidneys were more frequently affected in poisoning with the soluble salts of nickel especially when these were given intravenously.

VI. COMPARISON WITH IRON AND COBALT POISONING.

(i) *Iron carbonyl poisoning.*

As it is proposed to record the results of experiments conducted with iron carbonyl, pyrophoric iron and iron salts as well as with cobalt and its salts elsewhere, only brief reference to these experiments will be made in this place. It was found that 0.025 volume per cent. of iron carbonyl in air inhaled for 45.5 minutes (after deducting 14.5 minutes, see p. 567) killed rabbits. This yields a maximum quantity of iron of about 17 mgrs. It thus appears that iron carbonyl is less toxic than

nickel carbonyl. On the other hand, death takes place slightly more rapidly, the average length of life after poisoning with minimal quantities being 40·4 hours as against 69·3 hours after poisoning with nickel carbonyl.

The symptoms of iron carbonyl poisoning showed considerable similarity with those of nickel carbonyl. Beside the respiratory distress, dyspnoea, cyanosis, loss of weight and fall in temperature, coarse tremors were noted and also spastic gait and paresis of the hind extremities. The heart beat was frequently irregular and intermittency of the beat was noted. The physical signs of consolidation of the lungs were detected earlier than in nickel carbonyl poisoning. Diarrhoea was not present in any of the cases, and when recovery followed the poisoning, a peculiarity of the gait persisted for some time.

The pathological changes met with were of the same type as those of nickel carbonyl poisoning, but all the organs were affected without any preference. In the lungs, the pneumonic process was often intense and not infrequently enormous haemorrhagic infiltration was seen (see Plate VII, Fig. 3). No essential difference between the effects of the two carbonyls upon the lungs could be ascertained. The spleen, kidneys, stomach, intestines and other organs mostly showed haemorrhages and degeneration of parenchymatous cells, while the adrenals were only occasionally affected. The heart was generally dilated and haemorrhages were found in the walls. The brain also showed haemorrhages similar to those of nickel carbonyl poisoning.

The faeces were rarely dark in colour, save when the symptoms lasted for a long time, and diarrhoea was never met with. Iron was found in the faeces and urine. In the urine, it existed partly as a ferric and partly as a ferrous compound.

Comparative experiments with iron and iron salts were carried out in the same way as was done with nickel and nickel salts. The symptoms and pathological changes of iron and iron salt poisoning corresponded to those of iron carbonyl poisoning. The lethal dose of iron when given by intraperitoneal injection proved to be about 20 mgrs. per kilogram body weight. It could be shown that the ferric condition of the metal was retained in the excretion when a ferric compound was used, and the ferrous condition when a ferrous salt was introduced. Micro-chemical examination however showed that in the tissues, free iron ions were present, even if the compound introduced was in a condition of complex combination.

Cobalt was also studied in a similar manner. From these experi

ments, it could be shown that no essential difference between poisoning with nickel and poisoning with cobalt exists. On Plate VII, Fig. 4 the changes met with in the lungs after subcutaneous injection with cobalt chloride are shown, and from this it will be seen that all the characteristics of nickel poisoning are present. The only means of determining between the two conditions is by chemical analysis and by the appearance of the urine. The lethal dose of cobalt is higher than that of nickel, rabbits dying after the absorption of from 15 to 18 mgrs. per kilogram body weight, when applied intraperitoneally.

The urine of cobalt poisoning presents a peculiar colour. This was first described by Stuart. The first traces of the colour was seen within 15 minutes of the intraperitoneal injection of the tartrate of cobalt. As a rule, the colour attained its maximum during the course of the second day, but traces were seen during the following two or three days. The colour is a characteristic brown one. As this colour proved to be an easy index of the presence of cobalt in the urine, urine containing it was carefully studied. It was found that the quantity of cobalt varied in proportion with the intensity of the colour. When subjected to dialysis, the cobalt like the nickel passed very slowly through the membrane and for a considerable time during the dialysis a proportion was adsorbed into the membrane. The colour of the urine paled as the cobalt passed into and through the membrane. Boiling did not precipitate the metal. Lead acetate precipitated part of the cobalt but the total quantity could not be thrown out of solution by this means. Silver nitrate also precipitated part of the metal. In both cases, the colour became paler in correspondence with the amount of precipitation of metal. The cobalt containing urine did not form a sulphide on the addition of ammonium sulphide, but after boiling with nitric acid and neutralising with ammonia, the sulphide formed quite readily. 50 c.cm. of urine containing 17 mgrs. of cobalt was placed in two small cells connected with a syphon and a weak electric current was allowed to pass through the solution by means of platinum electrodes. After 24 hours, the syphon was clamped off in the middle, and the fluid in each half was emptied into the corresponding cell. The electrodes were washed with hydrochloric acid. It was found that the cobalt had not been ascertainably deposited on either electrode and that the quantity of metal was equally distributed between the two cells. A solution of cobalt chloride of the same strength was submitted to the same procedure. The cell corresponding to the negative pole contained over 10 mgrs. of cobalt while that corresponding to the positive electrode contained just under 7 mgrs.

The negative electrode was stained brown and 1.5 mgrs. of cobalt was dissolved off it, while the positive electrode was free from cobalt deposit. It thus appears that the cobalt in the urine is in a condition of complex combination and is not present as free cobalt ions. The behaviour of the urine is greatly in favour of the brown colour being due to a cobalt compound.

Pyrophoric reduced cobalt was suspended in serum and kept sealed up for many weeks, but no brown compound formed. When a cobalt salt such as the chloride is added to normal urine, no brown compound results and the cobalt can be combined with sulphur to form the sulphide, by adding ammonium sulphide without any previous heating with nitric acid. Several attempts were made to precipitate the compound or to crystallise it out of solution by the addition of various reagents, but without success.

VII. THERAPEUTIC EXPERIMENTS IN NICKEL CARBONYL POISONING.

It has been suggested that the administration of gases and other substances, which would dissociate nickel carbonyl and deposit nickel in an insoluble form, might lead to recovery after poisoning. The foregoing account however of the events occurring in nickel carbonyl poisoning makes it clear that attempts to convert the nickel compound into an insoluble one within the animal body will not alter the course of poisoning, as this has already taken place. It has been shown that even nickel sulphide is dissociated in the tissues and acts toxically. However it was considered necessary to conduct experiments in this direction, but the results coincide with the theoretical conclusions arrived at. Especial attention was paid to experiments aiming at the formation of the sesquioxide of nickel with bromine, as one of the assistants in Dr Mond's laboratories asserted that he always found relief in the inhalation of bromine after he had been exposed to nickel carbonyl vapour. No animals were saved by this treatment. The same may be said of chlorine vapour. The compounds used in these experiments included the acid phosphate of sodium, the triphosphate of sodium and the pyrophosphate of sodium, sulphuretted hydrogen and ammonium sulphide (poly-sulphides), the ferri and ferro-cyanide of potassium, the cacodylate of sodium and atoxyl, etc. In none of the experiments was a curative action discernible, and it must be assumed that either the more insoluble compound was not formed or that if it was formed it was again dissociated.

Better results attended a purely symptomatic treatment. The object aimed at was to keep the animals under the influence of narcotics and sedatives in order to slow down the circulation and respiration and thus to slow down the rate of absorption of the nickel. It was noticed that in some of the untreated animals, recovery had taken place when the animals remained quite quiet, while others which were apparently less severely affected died if they indulged in exercise during the early stages of poisoning. Morphine was found to be unsuitable. Veronal (diethyl malonyl urea) too did not lead to satisfactory results. A series of experiments with neuronal (bromine diethyl acetamine) showed that if given in doses just sufficient to keep the animals drowsy the fatal result could be averted in a considerable number, provided that only minimal lethal doses had been applied. When the dose of nickel was increased, the treatment failed to save any animals. Twenty-nine cats were exposed to 0.04 volume per cent. of nickel carbonyl vapour in air for 7.55 minutes. Sixteen were treated with neuronal and 13 served as controls. Of the treated cats 9 died and 7 recovered giving 43.7 per cent. of recoveries while of the controls 11 died and 2 recovered, giving 15.3 per cent. of recoveries. The drug proved to be quite harmless, which could not be said of veronal and morphine, and it was found that it was quite easy to regulate the dosage, so that the cats were only drowsy.

One is therefore justified, in the absence of a better form of treatment, to suggest that any cases of poisoning with nickel carbonyl should be treated by complete rest in bed, and small doses of neuronal (starting with 0.5 gram) repeated until somnolence begins to manifest itself.

Treatment only proved of value in cases when an amount of nickel carbonyl which was about a fatal dose had been inhaled, so that it is imperative to adopt preventive measures in places where nickel carbonyl is dealt with. That this is possible has been demonstrated by the fact that since 1904, no cases of poisoning have occurred at the Mond Nickel Works in Clydach, where nickel carbonyl is being produced in large quantities. At these works, the precautions taken to prevent escapes, and also the maintenance of a violent upward draught, effectually prevent an accumulation of poisonous vapour.

VIII. SUMMARY AND CONCLUSIONS.

Nickel carbonyl poisoning is a particular instance of nickel poisoning.

The lethal dose of nickel varies according to the method of application. When applied by subcutaneous injection, the physical condition of the compound influences the rate of absorption and therefore relatively large quantities may be required. In rabbits, the lethal dose is about $7\frac{1}{2}$ mgrs. per kilogram body weight under the most favourable conditions when applied subcutaneously. In cats it is about $12\frac{1}{2}$ mgrs. per kilogram body weight. When applied intraperitoneally, the absorbing surface is considerably larger and consequently the dose required to kill is smaller. In rabbits it is less than 7 mgrs. When applied in the form of nickel carbonyl vapour one meets with the most favourable conditions for rapid absorption and the dose is therefore still smaller. Rabbits die after the absorption of between 3 and 4 mgrs., while cats die after absorbing about $8\frac{1}{2}$ mgrs. per kilogram body weight.

In the lungs, nickel carbonyl is dissociated and a nickel compound, probably the hydrated sub-carbonate, is deposited on the respiratory surface.

The nickel is dissolved from the respiratory surface by the tissue fluids and is then taken up by the blood.

Some of the nickel finds its way directly through the lymphatic channels into the bronchial glands.

In the dissolved condition, the nickel enters into complex combination with some constituent of the body.

The nickel is carried by the blood to the tissues, but a selective absorption is exercised by the brain and adrenals. In the case of other forms of nickel poisoning, the lungs also exert this specific selection. The nickel only stays for a short time in these organs.

The specific pathological changes which are produced by nickel in these organs are primarily a degeneration of the endothelial cells of the capillary vessels. It is possible that some further primary action is exercised on the ganglion cells in the brain and on the parenchyma cells of the adrenals.

The haemorrhages follow as the result of the fatty degeneration of the vessel walls and secondary changes result from the effects of the haemorrhages.

The nickel is excreted by the kidneys and intestines.

The method of poisoning with iron carbonyl is similar to that of nickel poisoning, but the amount necessary to kill in the former case is larger.

Iron carbonyl poisoning like nickel carbonyl poisoning is merely a specific instance of metallic poisoning.

Iron acts in a similar manner to nickel on the walls of capillary vessels, but no evidence of selection by any special tissues was obtained.

Cobalt has a toxicological action which is identical to that of nickel. The lethal dose however is higher than that of nickel and lower than that of iron.

After the inhalation of a quantity of nickel or iron carbonyl which is greater than the minimum required to kill, no form of treatment was found to avert death.

It is with much pleasure that I again express my gratitude to Dr Ludwig Mond, F.R.S., for having rendered this investigation possible, by defraying all the expenses, and by giving me the benefit of his advice.

I further desire cordially to thank Dr C. J. Martin, F.R.S., and the other members of the Staff of the Lister Institute, who have at all times been ready and willing to assist me. The work has necessitated incursions into several branches of science, and has required the acquisition of a variety of methods. I have made free use of their kind collegiality and am glad to avail myself of this opportunity of recording my indebtedness.

The literature of the subject has been given in Part I, q.v.

DESCRIPTION OF PLATES.

PLATE VII.

Fig. 1. Section of lung of rabbit, poisoned by $\text{Ni}(\text{CO})_4$, showing intra-alveolar haemorrhage, oedema and fibrinous exudation; intra-bronchial exudation with proliferation of epithelial cells; cellular infiltration of the peribronchial tissue can also be seen. (Leitz Oc. 5, Obj. 3.)

Fig. 2. Same specimen under higher magnification. The oedema and haemorrhage are more clearly seen. Degeneration of the alveolar cells is present. (Leitz Oc. 3, Obj. $\frac{1}{12}$ oil immers.)

Fig. 3. Section of lung of rabbit poisoned with $\text{Fe}(\text{CO})_5$, showing enormous haemorrhagic infiltration. The alveolar structure has been partially broken up and the cells are degenerate. Some leucocytosis is present. (Leitz Oc. 3, Obj. $\frac{1}{12}$ oil immers.)

Fig. 4. Section of lung of guinea-pig poisoned by subcutaneous injection of CoCl_2 . Showing well-marked oedema and fibrinous exudation, inter- and intra-alveolar haemorrhage, with breaking up of the alveolar structure. (Leitz Oc. 1, Obj. $\frac{1}{12}$ oil immers.)

PLATE VIII.

Fig. 5. Section of supra-renal gland of rabbit poisoned with $\text{Ni}(\text{CO})_4$ vapour. Well-marked haemorrhagic infiltration and early degeneration of the nuclei of the parenchymatous cells. (Leitz Oc. 3, Obj. $\frac{1}{12}$ oil immers.)

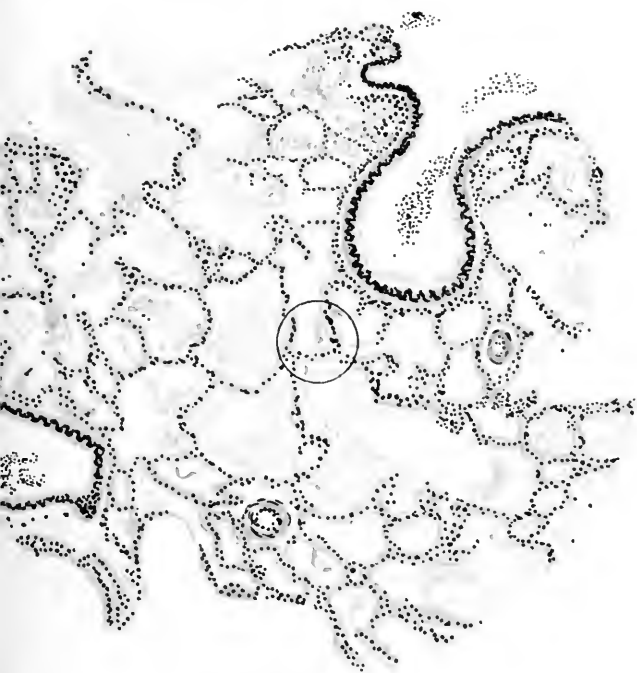
Fig. 6. Section of brain (close to the optic thalamus) of cat, poisoned by $\text{Ni}(\text{CO})_4$ vapour. Fixed by Marchi's osmic acid fluid and stained with haematoxylin and eosin. The specimen shows a small vein in transverse section. The wall of the vessel shows well-marked fatty degeneration. The fat globules are situated partly in the sub-endothelial space and partly in the endothelial cells. The latter are swollen, misshapen and poor in chromatin. (Leitz Oc. 3, Obj. $\frac{1}{12}$ oil immers.)

Fig. 7. Section of medulla oblongata of rabbit poisoned with $\text{Ni}(\text{CO})_4$ vapour, showing a capillary blood vessel giving way. The degenerative changes in the endothelial cells of the capillary wall are advanced; those cells which are still recognizable are swollen, indistinct and contain little chromatin. There is some leucocytosis in the neighbourhood of the capillary. (Leitz Oc. 3, Obj. $\frac{1}{12}$ oil immers.)

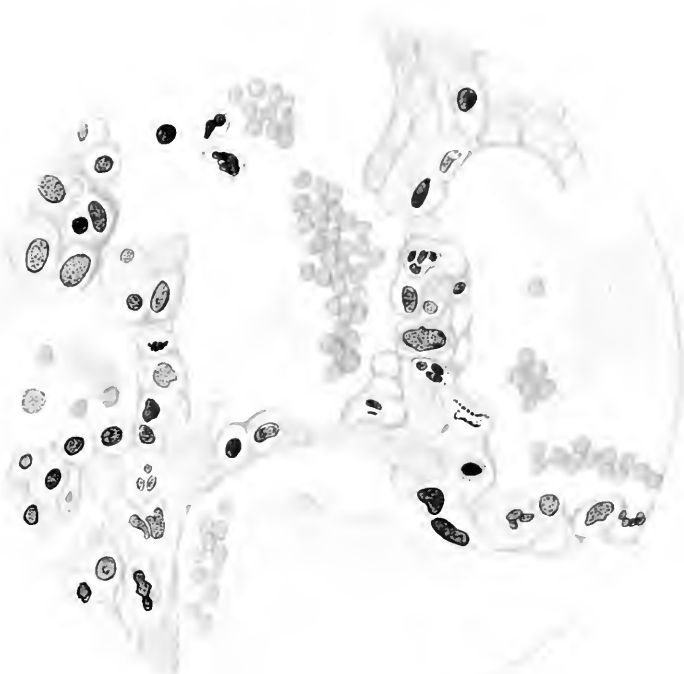
Fig. 8. Section of brain (mid-cerebrum) of rabbit poisoned with $\text{Ni}(\text{CO})_4$ vapour, showing chromatolysis of Nissl's granules in the ganglion cells. Many of the processes are broken off and the cells have become misshapen and have lost chromatin. Leucocytosis is also present. The vessel seen has not yet ruptured. (Leitz Oc. 3, Obj. $\frac{1}{12}$ oil immers.)

PLATE IX.

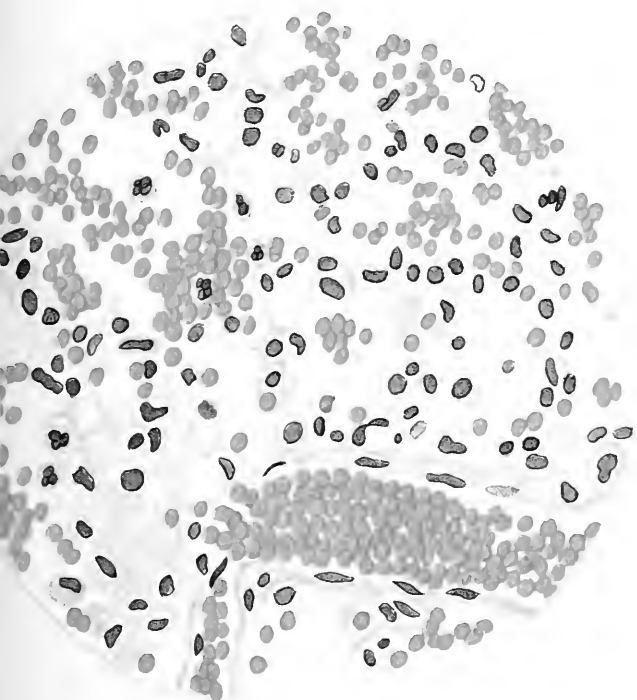
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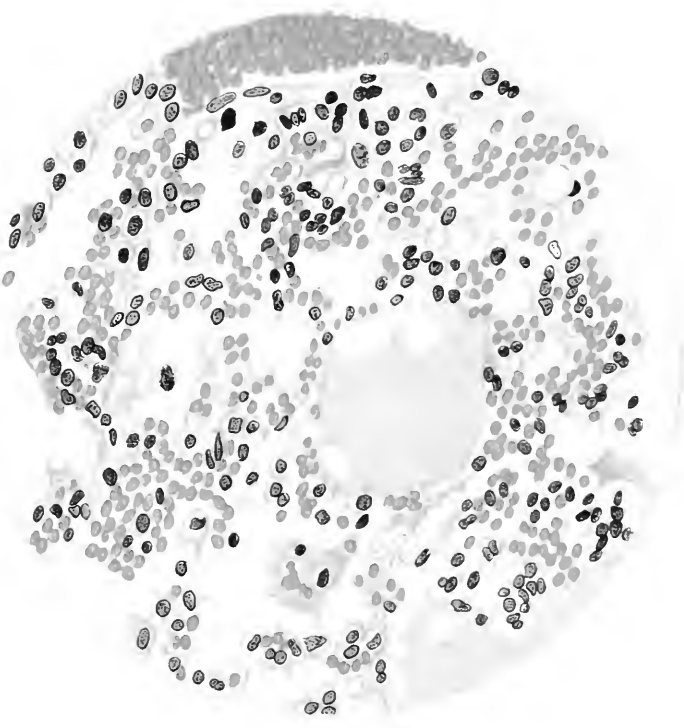
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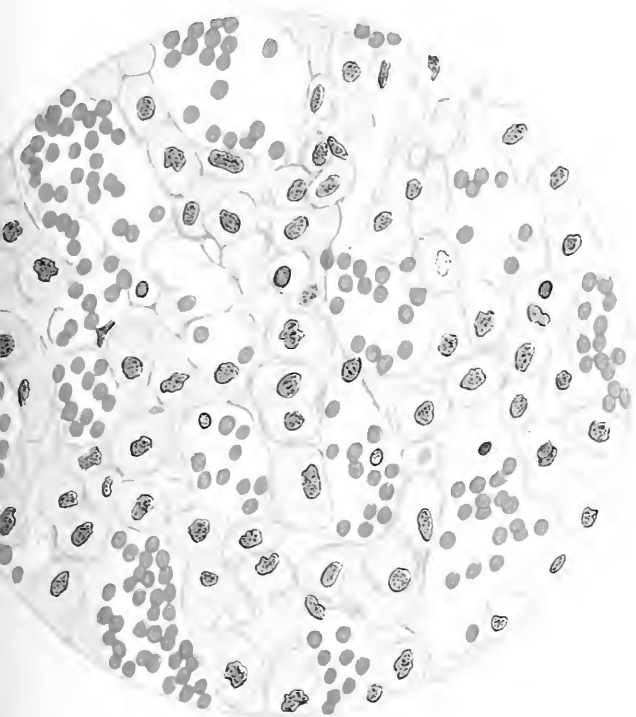
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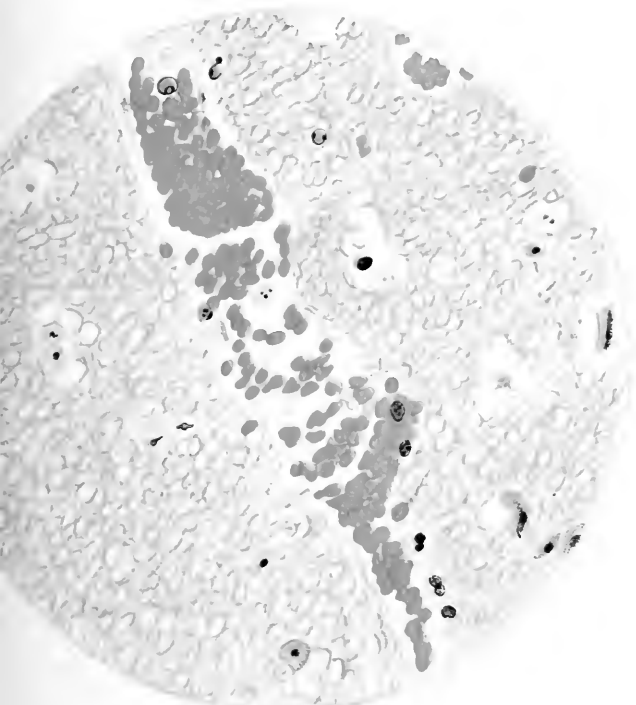
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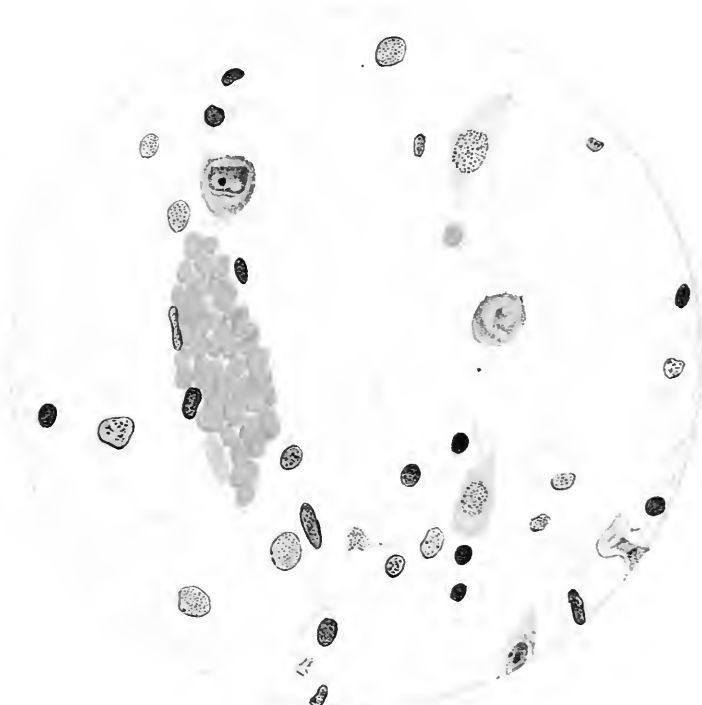
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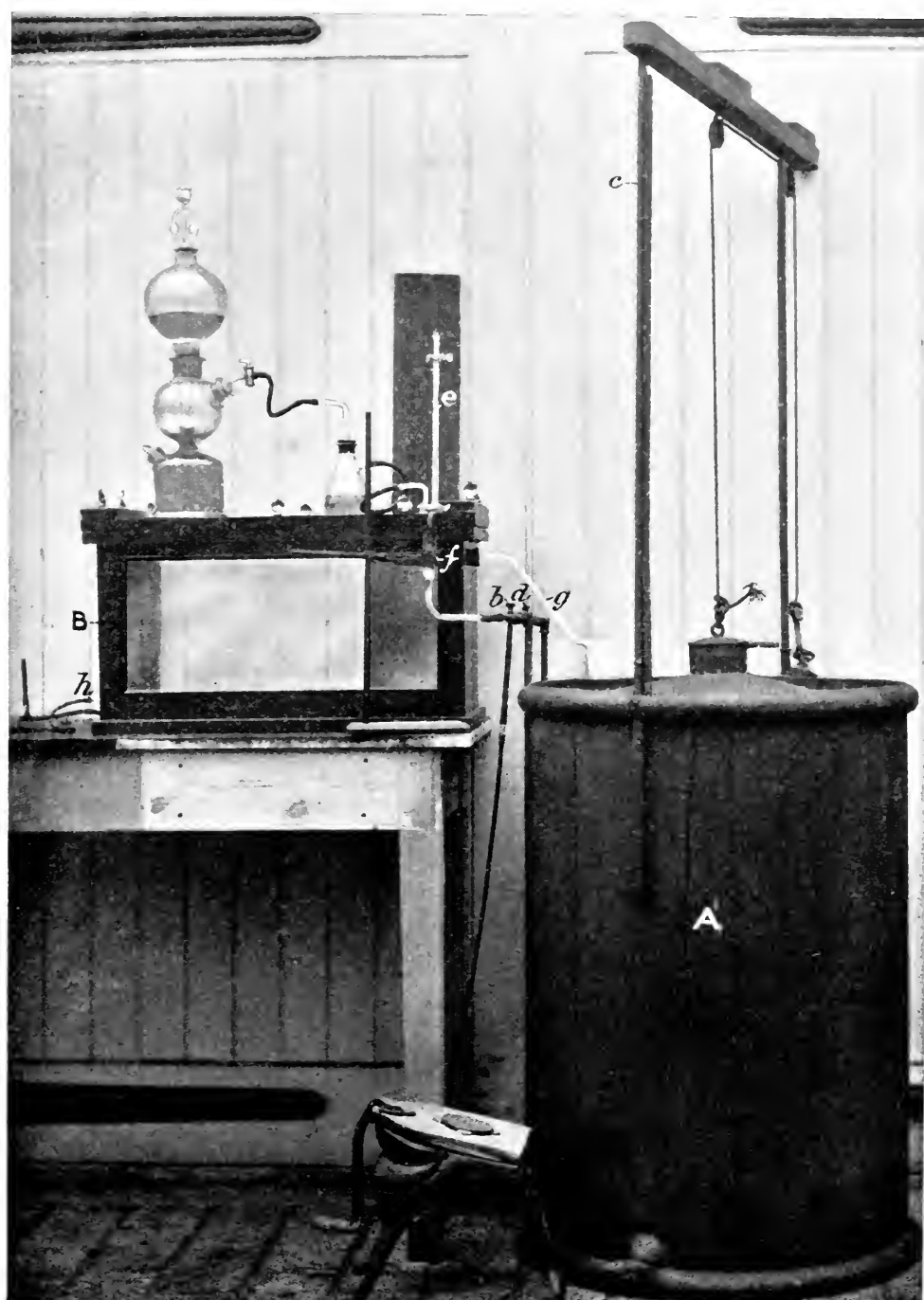
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8.



Apparatus used for Experiments
on Nickel Carbonyl.

AN OUTBREAK OF POISONING FROM INFECTED BRAWN.

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ALTHOUGH, during recent years, a good many food poisoning outbreaks have been described in the different Journals our knowledge as to the exact etiological factors is very deficient and it is still desirable that all outbreaks should be scientifically investigated and recorded.

The outbreak under consideration involved 18 cases with 3 deaths and occurred in May 1908 in the village of Murrow in Cambridgeshire.

Great difficulty was experienced in obtaining reliable particulars but the following was the general history of the cases.

On Friday, May 8th, 1908, Mrs J. B. of Murrow bought some pork bones, etc. from a local butcher in order to make pork-cheese (the local name for brawn) for the following week. This was a regular practice of hers. The pork-cheese was prepared the same night and finished the following morning. Then (May 9th) the pork-cheese contents were emptied out and, without cleaning the saucepan, potatoes and asparagus were cooked in it.

Mrs B., her son J. B. a young man of about 20 years of age and two little children all partook of the potatoes and the asparagus on the Saturday (9th) about midday, for dinner. All four became ill with diarrhoea and vomiting, the little children during the night, Mrs B. early on the Sunday morning (10th) and the grown up son J. B. about noon on the same day.

Mr B., the husband, the only other occupant of the house, was not at home for dinner and did not partake of the potatoes and asparagus. He remained quite well and unaffected.

Three pork-cheeses only were made and all three set up disease.

On Monday May 11th Mr B. gave one of the pork-cheeses (No. 1) to his neighbour Mrs D. The following morning Mrs D. and her two grand-daughters E. D. and J. D., all ate some of the pork-cheese for breakfast. E. D. was taken ill on Tuesday night (12th) about midnight, her sister J. D. about 8.0 p.m. Wednesday night and the grandmother (Mrs D.) early on Thursday morning, all with severe diarrhoea and vomiting. In these cases the incubation period varied from 16 to 44 hours. They were the only persons in the house.

Mr B. also made a present of a second pork-cheese to Mrs D. This untouched and the remainder of cheese No. 1 were handed over to Mrs R. and eaten by a third family, consisting of Mrs R., her five sons and one daughter M. R. All of them, except one child, made a meal of the pork-cheeses for supper May 12th and breakfast May 13th. M. R. was taken ill the evening of May 13th and Mrs R. and four of the sons during the 14th, all with diarrhoea and vomiting. The child who did not eat the brawn remained quite well.

The remaining pork-cheese (No. 3) was given by Mr B. on May 12th to Mrs W. This household consisted of five persons Mrs W., Mr W., their son R. W. and Mrs W.'s parents Mr T. and Mrs T. All five consumed the pork-cheese but the exact time is doubtful. All fell ill with diarrhoea and some with vomiting also. Mr T. was attacked Wednesday night (13th) and the others on the following day.

In all 18 persons were attacked and all of them consumed either the pork-cheeses or the vegetables cooked in the saucepan used to make the brawn. As far as we could ascertain in none of the cases was any of the brawn eaten by persons who remained unaffected.

The incubation period varied from about 12 to 48 hours. In some instances during this period no symptoms were noticed, in others faintness, nausea and pains in the stomach were complained of. The essential symptoms were diarrhoea and vomiting and were accompanied by headache, muscular weakness and prostration. In some these symptoms were very marked. The temperature varied from 100° to 103°.

Three of the patients died, a case mortality of 16·7 per cent., a very high percentage for such outbreaks.

The fatal cases were Mrs B. who died 20 days after eating the infected vegetables, M. R. who died 3 days after eating the brawn and Mr T. who died on May 19th probably about 5 days after the fatal meal.

A post-mortem examination was made in the first case by Dr R. H. Barrett, in the subsequent cases by one of us (C. H. G.) assisted by Dr Barrett. In the case of the child M. R. the post-mortem appearances were confined to the stomach and intestines which showed intense hyperaemia and inflammation.

In the other two cases the alimentary tract from the stomach to the rectum showed an almost continuous inflammatory condition, most marked at the pyloric end of the stomach, the duodenum, the caecum and the rectum. Adhesions between the different coils of intestine were also present. In the case of Mrs B. the lining membrane of the rectum was extensively ulcerated, the individual ulcers being about the size of a pea. The peritoneal adhesions were also much more marked and organised.

Bacteriological investigation. The remains of the pork-cheese were sent for chemical analysis and unfortunately none of the peccant material could be obtained for bacteriological examination.

Bacteriological investigations were not conducted in the case of the first two deaths, but in the case of Mrs B. who died on May 29th, portions of the internal organs were forwarded to one of us (W. G. S.), who bacteriologically investigated them.

From the spleen, kidney, bone-marrow and small intestine, the only organs examined, the bacillus described below was very readily isolated.

The characters of this "Murrow" bacillus were as follows:

A short bacillus with rounded ends. Decolourized by Gram's method of staining. Very actively motile. Grows very rapidly upon the ordinary laboratory media. Produces uniform turbidity in broth. Semi-translucent growth upon sloped agar. Bluish translucent growth upon gelatin without liquefaction. Produces no indole. In litmus milk produces first a small amount of acid followed by alkali production, the milk being slightly blue after 7 days and very markedly so after 9 days' incubation. In glucose neutral red broth produces fluorescence and reduction to orange colour. Ferments glucose, dulcitol, mannitol and maltose with production of both gas and acid. No fermentation of lactose, saccharose or salicin.

A mouse injected subcutaneously with 0.5 c.c. of a young broth culture was very ill with marked diarrhoea next day and died within 40 hours. The inoculated bacillus was readily isolated from the internal organs and also from the loose motions evacuated the day after inoculation.

A guinea-pig fed with a broth culture (10 c.c.) showed no ill effects.

The bacillus isolated is undoubtedly a member of the Gaertner group identical with or closely allied to *B. enteritidis*.

The inter-classification of the members of the Gaertner group can only be determined by agglutination or allied tests.

For this purpose the reactions of the bacillus under investigation towards the sera of animals immunized to a fairly marked extent by recognized members of the Gaertner group must be determined. Fortunately such sera were available prepared by one of us by inoculating rabbits with pure cultures of the respective bacilli. The three sera used were (1) paratyphoid serum, (2) *B. enteritidis* (Gaertner) serum, and (3) Aertrycke group serum, obtained respectively by inoculating rabbits with *B. paratyphosus* β isolated from a case of paratyphoid fever, with *B. enteritidis* isolated from a meat poisoning outbreak and with *B. meirelbeek* isolated by Van Ermengem from the Meirelbeek outbreak. (The last organism was obtained direct from Van Ermengem.)

The agglutination reactions of the Murrow bacillus and also of the immunizing bacilli to these sera are shown in the following table.

Serum and dilution		Murrow	<i>B. paratyphosus</i>	Meirelbeek	<i>B. enteritidis</i>
Paratyphoid	1 : 100	+	+	+	-
"	1 : 300	+		+	
"	1 : 500	-	+	+	-
"	1 : 1000	-	+	+ p	
"	1 : 5000		-	-	
Meirelbeek	1 : 100		+		-
"	1 : 300		+		
"	1 : 500	+	- a	+	-
"	1 : 1000	+	-	+	
"	1 : 5000	+		+	
"	1 : 10000	+ p		+	
"	1 : 20000			+	
"	1 : 40000	-		-	
<i>B. enteritidis</i>	1 : 100	-	-	-	
"	1 : 500	-	-	-	+
"	1 : 1000				+
"	1 : 5000				- a
"	1 : 10000				-

All microscopic tests in a hanging drop preparation ; time 2 hours.

+ p=partial and incomplete reaction only.

- a=no definite reaction but considerable action, i.e. a few scattered clumps.

From the serum reactions it is evident that the Murrow bacillus belongs to the Aertrycke or Meirelbeek sub-group and not to the sub-group represented by the *B. enteritidis* of Gaertner.

The proof that the Murrow bacillus was the cause of the outbreak was rendered nearly irrefutable by the results of a further series of tests with the blood of some of the sufferers.

It was only possible to obtain serum from three patients.

These were J. B. (of the first family attacked), Mrs R. and E. R. one of her sons.

The blood specimens were collected some weeks after the onset of the symptoms.

The results of the serum reactions are shown in the following table.

Serum and dilution		Murrow	Meirelbeek	<i>B. enteritidis</i>
Mrs R.	1 : 30	+	+	+
„	1 : 100	+	+	-
„	1 : 500	+	+	-
„	1 : 1000	-	-	-
J. B.	1 : 100	+	+	-
„	1 : 500	+	+	-
„	1 : 1000	+	+	-
E. R.	1 : 100	+	+	-
„	1 : 500	+	+	-
„	1 : 1000	+	+	-

All microscopic tests : time 2 hours.

This table shows very clearly that specific agglutinins were present for the Murrow bacillus and also for the *Meirelbeek* strain.

The reactions confirm the identity of the Murrow bacillus with Meirelbeek and show that the cause of illness in these cases was due to infection with this bacillus.

The complete proof, the finding of this bacillus in the peccant material was unfortunately impossible, but the details of the outbreak makes it abundantly clear that the bacilli were ingested in the brawn or in the vegetables, infected by being in contact with the brawn, in the uncleared saucepan.

All those who ate the pork-cheese or brawn became ill.

No persons were attacked except those who had either eaten the brawn or the vegetables cooked in the same vessel.

The question which next arises is, how did the brawn become infected? Did the Murrow bacillus infect this food before or after the cooking?

One of us (C. H. G.) has made very careful inquiries as to the methods of preparation of the pork-cheese. The method employed is to heat slowly and gently for many hours pigs-feet and any spare pork

bones in a saucepan with water. Towards the finish the bones are picked out, pepper and salt added and the whole remaining contents of the saucepan heated again and then poured into shapes. The method is one of slow heating and with a short boil at the finish. In this case the saucepan was allowed to stand on the side of the fire all night and the cooking finished the next day.

From the fact that the vegetables cooked after turning out the brawn became infected, it is very clear that the living bacilli were present in the brawn as present in the saucepan and that the infection was before and not subsequent to the preparation of the pork-cheeses. The fact that all the three pork-cheeses made were equally infective is also evidence as to the same fact.

It is quite clear, therefore, that in the present instance infected meat was used and that the so-called boiling which the food received was insufficient to kill the Gaertner bacilli in the meat, while on the other hand allowing the food to stand in the saucepan all night was probably a great factor in the multiplication of the organisms, a suitable temperature thus being afforded for the bacilli present to rapidly multiply in the highly nutrient material in the saucepan.

This great increase in the number of the bacilli makes it easy to understand that the contamination of the vegetables must have been gross and so have rendered the survival of some of the bacilli more probable. Possibly however the vegetables really were boiled and their infectivity may have been due to their being handled by utensils infected from the brawn and uncleansed.

In the present instance, unlike the majority of the English recorded outbreaks, it was possible to trace still further back the origin of the infection.

Careful inquiry elicited the fact that amongst the bones used to make this batch of brawn was a pig's-foot which appeared peculiar enough to cause comment upon it when Mrs B. saw it in the butcher's basket. This appearance the butcher attributed to the fact that on driving the pig to the starving-pen the foot was injured and he is reported to have said that although it could not be sold for prime meat it was all-right for cooking if she cut the injured portion out. We found it exceedingly difficult to obtain precise particulars, or indeed any information, as to the condition of this pig's-foot, the facts being concealed as much as possible. The nearest description we could get of it was that as purchased "it looked as if you had begun to cut a piece out and stopped short of doing so." It was also ascertained that the

injured pig was removed to the starving pen in a float and there kept for over 24 hours before being killed.

The facts are therefore not complete, but it is quite evident that some of the meat used was derived from a pig suffering from a local diseased condition. It may be that, as the butcher asserts, the local condition was the result of injury or, more probably from the description, that some local suppurative condition with abscess formation was present. In the latter case the condition was doubtless due to an infection with the Gaertner bacillus; in the former we must assume that the local condition offered a suitable nidus for infection by this bacillus derived from some unknown source.

The former is the more likely supposition and brings the condition in line with the findings in a number of the different German outbreaks.

The rest of the pig was also sold but no cases of illness traced to its consumption could be heard of, although careful inquiries were made.

Inquiries at the farm from which the pig came showed that no other pigs had been in any way ill, either locally or with diarrhoea and that there had been no cases of swine fever.

This last fact is of importance, since in cases of Swine fever or Hog cholera bacilli are present in the intestinal contents and elsewhere, which are of the Gaertner group and indistinguishable by their cultural characters from the bacillus isolated in this outbreak.

The relationship between paratyphoid fever and meat poisoning by Gaertner bacilli is at present undecided.

Two distinct views may be held. On the one hand it may be advanced that the two conditions are in no way related, and although both are caused by bacilli of the Gaertner group culturally indistinguishable yet the bacilli are really quite distinct and each can only set up its own pathological condition. On the other hand it is possible that the cases of meat poisoning due to bacilli of the Aertrycke type are caused by bacilli identical with *B. paratyphosus*, the different clinical picture being determined solely by different methods of infection, dosage, etc.

We do not propose to discuss these interesting questions here but they are mentioned since the course of the disease in Mrs B. is suggestive in this connection. The symptoms in this case were more like those of enteric fever and had it been an isolated case it would most probably have been diagnosed as that disease or if examined bacteriologically as paratyphoid fever. This patient died 20 days after the onset. The somewhat different relative prominence of the symptoms and the late-

ness of the fatal result were probably largely determined by dosage, the brawn itself not being eaten but only the vegetables cooked in the same saucepan. This case supports the view that the two conditions are closely related.

SUMMARY AND CONCLUSIONS.

1. Eighteen persons after eating a certain batch of pork-cheese, or vegetables infected from it, suffered from severe illness and three died.
2. The illness was caused by a Gaertner bacillus of the Aertrycke sub-group isolated from one of the fatal cases.
3. This bacillus was contained in the meat used for the pork-cheeses (brawn), infection being antecedent to preparation.
4. Part of the meat was obtained from a pig suffering from local injury or disease of one leg and the bacilli were no doubt etiologically connected with this condition.
5. The outbreak points to the need for a more complete and thorough veterinary inspection of meat before sale, and of the necessity for extended investigation into the diseases of animals used for human food caused by Gaertner bacilli.

ON THE RÔLE OF BACTERIA IN THE BIOLOGICAL
METHODS OF SEWAGE PURIFICATION, WITH SPECIAL
REFERENCE TO THE PROCESS OF DENITRIFICA-
TION¹.

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Introductory.

THE investigations described in the present paper were carried out
in connection with the experimental plant laid down by the Corporation

¹ Thesis presented for the Degree of M.D. of the University of Edinburgh, April, 1908.

of Belfast with a view to discover the most suitable method of dealing with the sewage of that city. The necessity of some form of sewage purification arises in Belfast in connection with what is known as the "foreshore nuisance" in Belfast Lough.

At the present time the sewage of Belfast is collected in two main sewers, a low level and a high level. At a first pumping station the whole sewage is collected in a reservoir which is discharged, during ebb-tide only, by a main outfall sewer carried well out into the Lough.

The upper reaches of the Lough are shallow, and at low tide large areas of mud, known as "sloblands" are exposed. On these mud banks there occurs during Spring and Autumn an enormous growth of green sea-weeds, chiefly *Ulva latissima* and various species of *Enteromorpha*. During late Summer and Autumn these sea-weeds are thrown up by storms on the shores of the Lough in large quantity where they decompose and give rise to a most disagreeable and penetrating stench which seriously affects the amenities of the whole surrounding district and no doubt has a deleterious influence on the health of those who are obliged to live in it. Letts (1900, 1901) has shown, by several different lines of argument, that the growth of the green sea-weeds in question is dependent on sewage contamination. *Ulva latissima* contains an unusually high percentage of nitrogen (6 % of the dried weed) and absorbs ammonia with great rapidity from sea-water when added either in the form of ammonium salts or as sewage. Whether these green sea-weeds utilise oxidised nitrogen in the form of nitrates to any large extent has not been definitely proved, but it appears generally to have been taken for granted that this form of nitrogen, which is the typical nitrogenous food supply of other plants, is also available for their growth.

The problem before the Belfast Public Health Committee therefore is, so to treat the sewage as to produce an effluent which has a minimum food value for the *Ulva* and similar sea-weeds, that is to say, an effluent containing a minimum of ammonia and as little nitrate as possible. It will be seen therefore that the object in view is rather different from that in most other towns where a highly nitrated effluent is considered desirable.

The experimental installation consisted in the first place of brick and coke "bacteria beds" for double contact treatment. Prof. Letts undertook the chemical examination of the sewage and effluents while Prof. Lorrain Smith investigated the matter from a bacteriological point of view. The purification effected by these beds averaged 57 % on the

total unoxidised nitrogen as estimated by Kjeldahl's method; this it will be observed is rather a low figure, and no nitrates or nitrites appeared in the effluents. Lorrain Smith (1901) showed that there is a constant decrease in the numbers of bacteria present in the effluent as compared with those in the original sewage, and that the beds which gave the best results chemically showed the largest reduction in bacteria. He also showed that the reduction in number of the bacteria was not due to exhaustion of the necessary pabulum in the sewage but that there must be other factors at work in the beds which lead both to the disappearance of nitrogen and to the destruction of the bacteria. Flasks of diluted nutrient broth inoculated with a loopful of sewage showed after five days a reduction of the nitrogen (Kjeldahl) of at most 12%, while sometimes the results were entirely negative. On the other hand, when the same broth was added to bricks removed from the contact bed a very striking disappearance of nitrogen occurred, amounting in some cases to 70% in three days. The bricks used were found to be covered with a sediment consisting largely of vegetable and animal organisms, and a copious layer of a similar sediment formed at the bottom of the flasks. This on analysis was found to be very rich in nitrogen. (It is clear that in these experiments processes of "adsorption" played a large part; this will be referred to later.) Lorrain Smith concluded that this layer of sediment on the bricks, consisting largely of animal and vegetable forms of life, was essential to the efficient working of the beds, and that a certain proportion of the nitrogen disappearing was built up into the bodies of these organisms. There was thus taking place in the beds a cyclical change in the forms of life such as occurs in a decomposing organic fluid, the later forms causing a more or less complete extermination of those appearing earlier in the series. "In this cycle of living forms, bacteria have an early place, owing, no doubt, to their power of rapid growth, and their turn to be exterminated comes correspondingly early also. In the contact beds a great process of extermination takes place. That this extermination is on a large scale may be inferred not only from the observed reduction in numbers, but also conversely from the overwhelming increase in numbers which appears in all the samples if they are kept in conditions favourable for bacterial growth. The effluent then represents the typical organic fluid at the point in the cycle of events when the bacterial forms are being exterminated. It is easy on this hypothesis to understand why the ratio of extermination of the bacteria should be in direct relation to the percentage of purification.

The bacteria, we may suppose, have absorbed into their bodies the greater proportion of the nitrogen available for food. They become in turn the food of infusorians which live in and form the sediment on the bricks, and so the change in forms of life proceeds. The cycle reached its final stage so far as I was able to observe with the introduction of worms (*Oligochaeta*). These would ultimately pass out to the Lough and become the food of fishes. The nitrogen by this indirect means passes away from the beds in the form of animal tissue. All the nitrogen which can be diverted to this end vanishes from solution, and the sewage is purified in proportion. To strike the cycle and measure the magnitude of one of its events is to measure the general capacity of this living economy for dealing with the available food nitrogen at any given stage, or indeed at all stages of its existence. To measure the ratio of extermination of bacteria therefore, is to measure the percentage of purification. Hence the correspondence between the two ratios." (Prof. Lorrain Smith's *Report*.)

On leaving Belfast in 1904 Prof. Lorrain Smith asked me to continue investigations on the bacteriology of the sewage and the effluents from the contact beds which had then been constructed on a larger scale, in order that bacteriological results might be available for comparison with the chemical analyses made by Prof. Letts.

It was desired in particular to determine to what extent the reduction in the numbers of bacteria is a measure of the chemical purification effected, and whether the different classes of bacteria are destroyed to the same extent. Later, a rotary "sprinkler" was constructed which gave a highly nitrated effluent. In connection with this, at Prof. Letts' suggestion, small experimental contact beds were used which were supplied with mixtures in varying proportions of the nitrated effluent from the sprinkler and the septic tank effluent. In these beds a large proportion of the oxidised nitrogen disappeared; they were therefore known as "denitrifying beds." This led to an investigation of the denitrifying bacteria in sewage and of their chemical action on nitrates in order to determine whether it would be possible to get rid of the nitrates in the effluent by their means. The combination of the sprinkler, which gives a highly nitrated effluent, with contact beds in which denitrification occurs, has thrown fresh light on the fate of the nitrogen which disappears in the process of purification by contact bed treatment.

There can now be no doubt that in this process a considerable amount of nitrogen escapes in gaseous form and an endeavour has

been made to get some idea of the relative proportion in which this occurs.

The present investigation therefore may be divided into two parts, viz.:

(1) An examination of the experimental contact beds, and of the sprinkler, with reference to the disappearance of certain groups of bacteria.

(2) An investigation of the occurrence of denitrification in sewage with reference to the bacteria concerned in the process, with isolation in pure culture of certain of these bacteria and a study of their chemical effects upon nitrates.

PART I.

(1) *The reduction in bacteria and its relation to chemical purification.*

The first series of observations were conducted during July and August, 1904, and consisted of an examination of the large double contact beds A, B, D, F and G. These were made up as follows:

Beds A :	Upper :	Medium Brick.
	Lower :	Fine ,,
Beds B :	Upper :	Medium Clinker.
	Lower :	Fine ,,
Beds D :	Upper :	Medium Coke.
	Lower :	Fine ,,
Beds F :	Upper :	Coarse Limestone.
	Lower :	Medium ,,
Beds G :	Upper :	Medium Limestone.
	Lower :	Fine ,,
“ Coarse ” signifies passed by 2½ in. mesh not by 1½ in. mesh.		
“ Medium ”	,, ,, 1½	,, ,, ½ ,,
“ Fine ”	,, ,, ½	,, ,, ⅓ ,,

The screened and sedimented sewage was treated in an open “septic tank” where it remained for about six hours and then on the contact beds, the period of each contact being three hours.

Full details of the structure and method of working these beds and of the other plant referred to in this paper are given in Prof. Letts’ *Third Report* (1908).

The samples of sewage at the various stages were taken as far as possible so that they are strictly comparable with one another. For

example, on August 5th a series of samples labelled as follows was received.

	Date	Time	
No. 1.	Aug. 4th	3.30 p.m.	Crude Sewage.
No. 2.	Aug. 4th	7.30 p.m.	Screened and Settled.
No. 3.	Aug. 5th	1.0 a.m.	Septic Tank Effluent.
No. 4.	Aug. 5th	7.0 a.m.	A. Lower Bed Effluent.
No. 5.	Aug. 5th	7.0 a.m.	B. „ „ „
	etc.	etc.	

A similar set of samples was examined each time. The samples were received at the laboratory at 10 a.m. and the examination begun at once. Five examinations were made of this series on the following dates: July 22nd and 26th, August 5th, 12th, and 23rd. The following groups of bacteria were estimated:

- (1) Those growing on Gelatin at 22° C.
- (2) Those growing on Agar at 37° C. (three examinations).
- (3) Spores growing on Gelatin at 22° C. (aërobic).
- (4) *Bacillus coli*.
- (5) *Streptococcus*.
- (6) *Bacillus enteritidis sporogenes* (Klein).

Attention was paid to the three last named bacteria, first, because their presence is easily detected, and secondly, because *B. coli* and *Streptococcus* are characteristic intestinal organisms while *B. enteritidis*, as an anaërobic spore-bearing bacillus, represents a class not otherwise taken into account in the investigation. The first three groups were counted by the ordinary plate methods. The dilutions were made by means of pipettes delivering 1 c.c., flasks of about 250 c.c. capacity containing 100 c.c. of water, and test-tubes containing 9 c.c. of water. A large supply of these in a sterile condition was prepared before each examination of a series of samples. The flasks enable the high dilutions required to be rapidly attained, and have the further advantage of allowing very thorough shaking so as to secure a uniform distribution of the bacteria. For the counting of spores the tubes containing the necessary dilutions were exposed to a temperature of 80° C. for ten minutes to kill off the other forms.

After some preliminary examinations it was found that the dilutions required to give satisfactory plates for enumeration were as follows:—

Crude Sewage, Screened Sewage, and Septic Tank.

For gelatin plates:	1/1,000,000	and	1/10,000,000.
For agar	„	1/100,000	„ 1/1,000,000.
For spores	„	1/10	„ 1/100.

Contact Bed Effluents.

For gelatin plates: 1/100,000 and 1/1,000,000.

For agar ,, 1/10,000 ,, 1/100,000.

For spores ,, 1/10 ,, 1/100.

Two plates were made with 1 c.c. each of each dilution, and where possible only plates with more than five and less than a hundred colonies were counted. The agar plates were incubated at 37° C. for 24 hours; the gelatin plates at 22° C. for two days.

B. coli and streptococci were estimated by the following method which is a modification of that described by Prescott and Winslow (1904) and depends upon the fact that when these two organisms are grown together in glucose broth, as the medium becomes acid the streptococci outgrow the bacilli and after two days can be easily isolated from the mixture by means of litmus lactose agar plates. On this medium the streptococcus colonies are small and intensely red, and are easily distinguished from the larger and less acid colonies of *B. coli*. One cubic centimetre of the several dilutions of the sewage was added to glucose broth in Durham's fermentation tubes. The tubes were incubated at 37° C., and on the following day those which showed gas formation were noted, and a loopful from each was smeared on the surface of a gelatin plate, the tubes being returned to the incubator. After two days at 22° C. these gelatin plates were examined and non-liquefying colonies (where possible those showing the typical "vine-leaf" structure) were picked out and tested in pure culture. The additional tests used for the identification of *B. coli* were, gas formation in glucose broth, coagulation of milk, and indol formation in peptone water.

For streptococci the same glucose broth tubes were examined after incubation at 37° C. for two days. It was usually sufficient to examine a loopful in a hanging drop preparation under the microscope for the presence of chains of streptococci.

The "*B. enteritidis* test" was done as follows: 1 c.c., 1/10 c.c., and 1/100 c.c. of the samples were added to deeply filled whole milk tubes which were first heated to 80° C. for ten minutes and afterwards incubated at 37° C. for twenty-four hours. Clotting of the milk, with gas formation and tearing of the clot, were regarded as evidence of the presence of organisms of this class.

Of course these methods only give a rough approximation to the

number of bacteria of the classes named which are present; thus if the presence of *B. coli* be demonstrated in 1/100,000th of a cubic centimetre, but not in a millionth, we may conclude that there are at least 100,000 but not so many as a million present per c.c. and in the tables the fraction 1/100,000 is used to indicate this. If a number of tubes be inoculated from the same sample a closer approximation may be obtained; thus if *B. coli* be found in 5 tubes out of 10 inoculated with 1/100,000 c.c. we conclude that the number present in 1 c.c. is about 500,000. An approximation to the average number in a large series of examinations may be made in the same way, and the averages for *B. coli* and streptococci given in Table I are calculated on this principle.

The detailed results of the examination of each sample are given in Table I, and the average for each sample noted for the bacteria growing on gelatin and on agar and for spores.

The proportion of bacteria growing at body temperature to those growing at 22° C. is nearly 1 to 10 in the untreated sewage and in the effluent from bed A, while it is as 1 to 5 in the other effluents.

The spores are remarkably few in number, varying from 2000 per c.c. in the crude sewage to 90 per c.c. in effluent A. This may be taken to indicate that the sewage is a favourable medium for bacterial growth.

The presence of *B. coli* and streptococci was demonstrated in nearly every case in 1/100,000 c.c. and occasionally in 1/1,000,000 c.c. both in the untreated sewage and in the effluents.

The constant decrease in the numbers of *B. enteritidis* during the period of observation is remarkable. On July 22nd this organism was present in 1/1000 c.c. of the crude sewage, while on August 23rd it could only be found in 1 c.c.

A calculation of the average numbers of *B. coli*, streptococci, and *B. enteritidis*, is made for the three samples of untreated sewage, and for all the effluents respectively. The actual numbers given are probably too high, but they serve to indicate the percentage reduction for these classes.

There is a progressive diminution in the numbers of bacteria during the whole process of purification; they are reduced to about one half in the screened and settled sewage, and again to about one half in the "septic tank." It should be noted in connection with this latter fact, that the sewage was not subjected to a closed septic tank treatment. The tank was open, and the process took more the form of a further sedimentation than anything else.

The greatest reduction in numbers of bacteria occurs in the contact beds.

This is best seen in Table II, where the percentage reduction in the various classes is shown, calculated on the crude sewage, and also (for the effluents) on the septic tank.

The corresponding chemical results (average of two examinations on July 22nd, and July 26th, by Prof. Letts) are given for comparison.

It will be observed that the effluent from bed A, which shows the best chemical results, especially as regards loss of free ammonia, also shows the greatest reduction in bacteria of all classes. The various beds may in fact be arranged in the same order of efficiency whether they are judged by the chemical or by the bacteriological standard. There can be no doubt therefore that the decrease of bacteria in the effluent is an exact measure of the chemical efficiency of a contact bed. The superiority of bed A is most marked when judged by the destruction of bacteria growing at 37° C. and of spores. It would appear therefore that most attention should be paid to these groups in measuring the efficiency of a bed by bacteriological methods.

With regard to *B. coli* and *Streptococcus* it should be noted that these are proportionately less reduced in number than the other groups; and if these organisms be taken to represent the pathogenic class, there is no evidence in the above results to show that this class is destroyed in the "bacteriological" methods of sewage purification, as has sometimes been supposed.

With reference to the cause of the disappearance of the bacteria shown in the above enumerations it must be observed that the reduction begins in the sedimenting sewage and continues in the so-called "septic tank." It appears probable that here the process is a merely mechanical one, due to sedimentation, for we can hardly believe that higher forms of life are at work destroying the bacteria under such conditions. But the reduction in numbers of bacteria in the same period of time is much greater in the contact beds. This fact is important, and affords clear evidence that other than purely mechanical factors are at work in causing the disappearance of bacteria in the beds. That these factors are essential to the efficient working of the beds is shown by the correspondence of the chemical and bacteriological results.

Included in Table II are the results of one examination of the effluent from the rotary sprinkler, this effluent being in every way comparable with those from the contact beds. The reduction in bacteria is much greater than that shown by the best of the contact

beds. This is in agreement with the results of the next series of examinations, which will now be given.

This consisted in a bacteriological examination of the effluent from the sprinkler and from the denitrifying contact beds used in connection with it, as compared to the septic tank effluent. Six observations were made during January and February, 1905.

The sprinkler was constructed in segments of different materials corresponding to those used in the contact beds already described. It was worked intermittently by an automatic arrangement and was supplied from the septic tank. The denitrifying beds were of small size, were made up of bricks similar to the A beds, and were worked in a similar fashion. They were supplied with a mixture of equal parts of the sprinkler and septic tank effluents during the period of the earlier examinations. On the dates of the two last examinations they were receiving the sprinkler and septic tank effluents in the proportion of 3 : 4. The lower denitrifying bed for second contact was started early in February and was only examined on two occasions.

The same technique was employed as before, and the same groups of bacteria were estimated (with the exception of spores and *B. enteritidis*).

Corresponding chemical analyses, made by Prof. Letts, are available for the whole series, and are given in Table IV. The detailed results of the examinations on the different dates are given in Table III, and also the averages for the whole series. The number of bacteria in the mixture of sprinkler and septic tank effluents are calculated for each date, and given in the table for comparison with the effluents from the denitrifying beds. Table V shows the percentage reduction in the number of bacteria and the percentage chemical purification calculated for the septic tank, and in the case of the denitrifying beds, for the mixture of septic tank and sprinkler effluents.

In comparing the results with those of the first series it must be remembered that in this case the crude sewage was not examined, and the results calculated for the septic tank in the first series must be taken for comparison.

The numbers of bacteria are rather lower than in the first series, corresponding, no doubt, to the different season of the year. The proportion of those growing at 37° C. to those growing at 22° C. is, as before, about 1 : 10, both in the sewage and in the effluents. There is again a general correspondence between the chemical and the bacteriological results. In the sprinkler effluent there is a very large reduction

in the number of bacteria, all the groups being about equally affected. This reduction is considerably greater than that shown by the best of the contact beds (A) in the previous series. The averages are given here for comparison.

Reduction in Bacteria (on Septic Tank).

	22° C.	37° C.
Sprinkler	93.4 %	96 %
A beds	69 %	63 %

This does not correspond to any greater efficiency of the sprinkler from a chemical point of view, as is shown by the following figures.

Chemical Purification (on Septic Tank).

	Free NH ₃	Alb. NH ₃	Oxy. abs.
Sprinkler	71.5 %	51 %	63 %
A beds	89 %	53 %	72 %

On the other hand there is very little reduction in the bacteria in the upper denitrifying bed, although there is considerable chemical purification (Table V).

These contrasted results show that the reduction in bacteria is not a measure of the chemical purification when processes so different as those at work in the sprinkler, in the denitrifying bed, and in an ordinary contact bed, are in question. This is what we might expect when we remember that the destruction of bacteria is not an essential factor in the purification, but only a usual accompaniment, and an indication of the activity of higher forms of life.

If however we compare the effluent from the lower denitrifying bed with that from the A beds of the first series, we find that the chemical and bacteriological results are practically similar.

Reduction in Bacteria.

	22° C.	37° C.
A beds	69 %	63 %
Denitrifying bed	72 %	87 %

Chemical Purification.

	Free NH ₃	Alb. NH ₃	Oxy. abs.
A beds	89 %	53 %	72 %
Denitrifying bed	76 %	55 %	67 %

The amount of nitrate in the two effluents also corresponds (about 0.25 parts N. per 100,000).

This gives us grounds for believing that, on the whole, the same processes are at work in the double contact beds on the one hand, and in the combination of sprinkler and denitrifying beds on the other. It will be shown in the second part of this paper that in the process of denitrification nitrogen escapes into the atmosphere in the form of gas. The facts now before us indicate that in the process of treatment by double contact successive stages of nitrification and denitrification occur, exactly comparable to the stages represented by the sprinkler and the denitrifying beds. When the contact beds are empty, in the so-called "resting" stage, active oxidation takes place, and among other changes of a similar nature, ammonia is oxidised to the form of nitrate. When the bed is again filled, it acts as a "denitrifying bed," and a large proportion of the oxidised nitrogen disappears in gaseous form. This view is confirmed by a general consideration of the physical and chemical processes which occur in "contact beds" and in "percolating beds" (such as the sprinkler). It will be convenient to give here a short account of our present knowledge of these processes.

(2) *Physical and chemical processes in "contact" and "percolating" beds.*

We owe our knowledge of this subject chiefly to the work of Prof. Dunbar, Director of the Hygienic Institute in Hamburg. Dunbar (1900) has shown that the purification of sewage by the artificial biological methods depends very largely on processes of "adsorption." This term is used to indicate a little understood process of a physical nature whereby organic substances in solution are thrown out of solution, or retained in a more concentrated form, in the interstices, or on the surface of solid substances of a suitable nature, when these are immersed in the solution in question.

Thus if pieces of filter paper are placed in a dilute watery solution of a stain, they take up more of the colouring matter than of the water, and the solution in consequence gradually becomes less and less coloured. This is a simple but sufficiently accurate illustration of the process of "adsorption." No distinction is here made between "adsorption," which strictly refers only to surface action, and "absorption" in its limited sense, as this latter term is apt to give rise to confusion from its more common and wider use.

Working at the question from an entirely different point of view, Dunbar confirmed Lorrain Smith's view as to the inadequacy of the purely bacterial theories of sewage purification which had previously been in vogue. He showed that the material used in the construction of the beds, e.g. clinker, is possessed of very considerable adsorptive properties, and that these are very much increased by the slimy deposit which forms on its surface in a ripe bed. Dunbar showed further, by means of small experimental beds, that a sudden great reduction in the amount of organic matter in the sewage takes place within five minutes of the filling of the bed, and that this reduction is only slowly increased on further contact. He also demonstrated adsorption effects by the use of methylene blue, the effluent in this case remaining permanently decolourised, whereas bacterial action does not produce permanent decolouration. The adsorptive powers of a bed are very rapidly exhausted unless it is allowed intervals for rest and recovery. Dunbar showed this by the following experiment. A small contact bed was filled with sewage and allowed to stand for one hour; it was then emptied and filled again five times in rapid succession, and the various effluents gave the following results on analysis.

	Purification	Odour
After standing one hour	56.2 %	Earthy.
1st filling	65.0	"
2nd "	66.2	"
3rd "	32.0	Faintly faecal.
4th "	27.3	Faecal.
5th "	16.5	"

There was here a rapid falling off in the purification effected after the second filling.

Dunbar also showed the importance of the resting interval by another set of experiments. In these the amount of oxygen taken up from the atmosphere, and the amount of carbonic acid produced in the bed, were estimated, and were found to be much greater when the bed was empty than when filled.

The most remarkable fact about a contact bed is its great power of recuperation when allowed a sufficient interval for rest. Its adsorptive capacity is rapidly re-established, and this recovery is accompanied by active chemical changes largely of the nature of oxidation. From this point of view the bed shows most activity during its so-called resting period.

The organic material in solution is retained in the bed by adsorption, that in suspension is no doubt retained also by physical processes of a

simpler nature; when the bed is emptied and freely exposed to air, the retained organic material is subjected in a concentrated form to the attack of the various forms of life in the bed, and it is owing to this vital activity that the bed is able to regain its absorptive powers. That the activity of these vital processes is greatest when there is a free supply of air is in accordance with the view that it is chiefly the higher forms of life which are at work. No doubt the bacteria also play an important part in breaking down complex organic substances, and in other respects. If these various forms of life are not allowed to perform their scavenging functions the absorptive powers of the bed are speedily exhausted, and purification ceases.

It is probable that some substances in solution in the sewage are retained in the beds by purely chemical means, independently of adsorption. Thus, as Dunbar (1903) points out, sulphuretted hydrogen combines with iron to form sulphide, which is then oxidised to ferric sulphate. This is precipitated by the alkaline sewage as hydrate, in which form it remains in the bed.

It appears probable also that the adsorptive functions of the bed do not cease when it is emptied. The enormous oxidising power of the empty bed is materially assisted by the presence of adsorbed or condensed oxygen which is more potent chemically than ordinary oxygen. Thus Lübbert has shown that dimethylanilin, when in contact with clinker, is oxidised in air to methyl violet. This reaction does not occur on exposure to air under ordinary conditions; it is commonly effected by heating with potassium chlorate.

In percolating beds like the sprinkler these various processes of absorption and oxidation with resulting purification take place simultaneously. It is quite impossible to understand the very rapid effect produced by such beds apart from the adsorption theory. The sewage leaves the bottom of the bed in a purified condition a very short time after its delivery at the top. The organic material has simply been retained in the bed for future purification. If this is satisfactorily effected the adsorptive powers of the bed are not exhausted and the process of purification goes on continuously. The reduction in the number of bacteria must be regarded from a similar standpoint. These are at first simply retained in the bed by a process of filtration. They are there destroyed in large numbers; otherwise the capacity of the bed for acting as a bacterial filter would soon become exhausted.

PART II.

STUDIES ON DENITRIFICATION.

(1) *Introductory.*

It has long been known that nitrates, in contact with certain organic substances, are destroyed with evolution of nitrogen gas and gaseous oxides of nitrogen. This process is known as denitrification. It is in marked contrast to the opposite process of nitrification, whereby nitrates are formed from ammonia, or, under certain conditions, even from free nitrogen. The latter process is essential for plant life; the former if allowed to proceed unchecked would render plant life impossible. Both processes have been shown in recent years to be due to bacterial action. Denitrification has been studied chiefly in connection with agricultural problems. The literature is already very extensive, and cannot here be referred to in any detail. Full references to it are given in an article by Jensen (1904) in Lafar's *Handbuch der technischen Mykologie*. In that article Jensen divides the bacterial processes which are accompanied by a reduction of nitrates, under the following heads:

- (1) Reduction of nitrates to nitrites and ammonia.
- (2) Reduction of nitrates and nitrites to lower gaseous oxides of nitrogen (N_2O and NO).
- (3) Reduction of nitrates and nitrites with evolution of free nitrogen.

With regard to (1) it is well known that a very large number of bacteria have the power of reducing nitrate to nitrite; it is however doubtful whether in any case ammonia is formed by the reduction of nitrate.

It is chiefly with the processes mentioned under (2) and (3) that we are here concerned. Strictly speaking the term denitrification should be confined to (3).

With regard to the production of lower gaseous oxides of nitrogen little work has been done, and no pure cultures have hitherto been described which reduce nitrate to this stage only.

The typical denitrifying bacteria reduce nitrate or nitrite with formation of free nitrogen gas. When pure cultures of these bacteria are grown in peptone broth containing 0.25 % potassium nitrate or nitrite a very characteristic foaming of the culture occurs owing to the production of gas.

In 1886 Gayon and Dupetit isolated two species of denitrifying bacteria but did not describe their characters sufficiently to allow of identification. When one of these was grown in nitrate broth containing asparagin both nitric oxide (NO) and free nitrogen were produced; in the absence of asparagin only free nitrogen occurred.

The first thorough bacteriological investigation we owe to Burri and Stutzer (1895), who isolated from horse dung two bacteria which they called *B. denitrificans* I and II. These were afterwards renamed by Lehmann and Neumann, *B. denitrificans*, and *B. Stutzeri*, respectively. While *B. Stutzeri* could decompose nitrate with gas formation in pure culture, *B. denitrificans* could only do so when accompanied by *B. coli*. The explanation of this fact was given later by Weissenberg (1897), who showed that *B. denitrificans* can attack and decompose nitrite but not nitrate. Since *B. coli*, in common with many other bacteria, has the power of reducing nitrate to nitrite, it is easy to understand how a combination of these two bacteria can attack nitrate and destroy it with formation of free nitrogen.

Since then a number of other denitrifying bacteria have been isolated and described, and it has been shown that certain bacteria previously known, e.g. *B. pyocyaneus*, possess this property.

(2) *Denitrification in Sewage.*

In 1904 Letts showed that when potassium nitrate was added to septic tank effluent in a proportion equivalent to 2.5 parts of nitric nitrogen per 100,000 parts of mixture, the nitrate disappeared in 24 hours, and in four out of eight experiments the theoretical amount of nitrogen could be recovered in the form of free nitrogen and nitric oxide, the latter being present only in small amount. In one experiment where the septic tank effluent had been previously filtered through porcelain the disappearance of nitrate did not occur.

Prof. Letts also found that if equal parts of septic tank and sprinkler effluents were mixed and allowed to stand in a stoppered bottle, the nitrate disappeared in from one to two days. It occurred to him that this process might be hastened if the mixed effluents were treated in a contact bed; for this reason the denitrifying beds already described were constructed, and it was in fact found that the nitrate disappeared almost completely after three hours contact with the bed. This is shown in Table IV. On Jan. 25th, the nitrate was reduced only from 1.32 to 0.82, but at this date the bed was new; on the subsequent

examinations complete disappearance of the nitrate occurred with the exception of that on Mar. 1st, when only 90 % reduction was found, the amount of nitrate in the sprinkler effluent on this date being exceptionally large. After treatment on the lower "denitrifying bed" a certain amount of nitrate again appeared in the effluent.

Although the single observation of Prof. Letts, mentioned above, in which the sewage was filtered through porcelain, appeared conclusive as to the bacteriological nature of the process of denitrification in sewage, it was thought advisable to confirm this by other means. The following experiments were therefore carried out.

Preliminary experiments to show that denitrification is due to bacterial action.

Test tubes and flasks plugged with cotton-wool were used, no attempt being made to secure anaërobic conditions. The temperature, except where otherwise stated, was 37° C. By "nitrate reaction" the Brucin Sulphuric acid test is to be understood; this of course indicates the presence of either nitrate or nitrite.

Sp. = Sprinkler effluent. S. T. = Septic tank effluent.

(1) 5 c.c. Sp. + 5 c.c. S. T. in test tube: complete disappearance of nitrate reaction in 24 hours.

(2) 10 c.c. Sp. in test tube: no diminution in nitrate reaction in 24 hours.

The sprinkler and septic tank effluents were now filtered through porcelain, tested, and found to be sterile.

(3) 5 c.c. filtered Sp. + 5 c.c. filtered S. T.: no diminution in nitrate reaction in 48 hours.

(4) 5 c.c. filtered Sp. + 5 c.c. filtered S. T. + loopful of unfiltered S. T.: complete disappearance of nitrate reaction in 48 hours.

These experiments show that denitrification is due to the action of micro-organisms which do not pass through a porcelain filter.

A dilute peptone broth (1 in 20 of the ordinary nutrient broth) was now prepared with the addition of 0.03 % potassium nitrate. This contained 2 parts of nitric nitrogen in 100,000, or rather more than the average amount of nitrate in the sprinkler effluent.

(5) Two flasks (capacity 250 c.c.) containing 100 c.c. of this dilute nitrate broth each received 1 c.c. S. T. effluent. One flask (A) was kept at 37° C., the other (B) at 22° C.

	Nitrate reaction	
	A	B
After 24 hours	Positive	Positive
After 2 days	Negative	Positive
After 3 days	Negative	Positive
After 4 days	Negative	Negative

This experiment shows that denitrification occurs more readily at the higher temperature, and confirms the previous results as to the bacterial nature of the process.

(3) *The Denitrifying Bacteria isolated.*

The isolation of denitrifying bacteria was carried out in the first place by means of a dilute nitrite peptone broth, corresponding in composition to the dilute nitrate broth mentioned above.

A flask containing 100 c.c. of this dilute nitrite broth was inoculated with 2/10 of a c.c. S. T. effluent. After 24 hours at 37° C., when the nitrate reaction had disappeared, gelatin plates were made; at the same time another flask was inoculated with a loopful from the first, and after 24 hours a loopful from this was inoculated into a third flask, from which gelatin plates were also made. When colonies appeared on the gelatin plates they were isolated and tested in nitrate and nitrite broth. There appeared to be no advantage in carrying on successive inoculations from one flask to another, as more denitrifying colonies were found on the first set of plates than on the second. Two species of denitrifying bacteria were found. These are described below as Nos. 1 and 2.

In the course of further investigations into the number of denitrifying bacteria present in the various samples of sewage, pure cultures were isolated and tested from time to time. *B. pyocyaneus* was thus found on several occasions, and an additional new species, described below as No. 3, was found.

The bacteria isolated may be divided into two groups, according to whether or not they produce gas in 0.25% potassium nitrate broth. Those which do so belong to the class of typical denitrifying bacteria. All the bacteria isolated were able to reduce nitrate in pure culture; thus bacteria belonging to the class represented by *B. denitrificans* (L. and N.), which are able only to attack nitrite, were not found.

A. *Bacteria which produce gas in 0.25% potassium nitrate broth :
the typical Denitrifying Bacteria.*

Bacillus No. 1. This bacillus was isolated on more than one occasion. It appears to be identical with *B. Stutzeri* (L. and N.).

It is a motile non-sporing bacillus, resembling *B. coli* in hanging drop and in stained preparations. It does not retain the stain in Gram's method.

Gelatin stroke culture: A hard dry wrinkled growth along the needle track, slightly yellowish in tint. No liquefaction of the gelatin.

Gelatin plate culture: The surface colonies after two days are round or irregular, elevated and crenated at the margin, and so hard and tenacious that it is difficult to obtain subcultures without lifting off the whole colony. The deep colonies are round and not characteristic.

Agar stroke culture: The growth is hard, dry, and adherent, white in colour, and not very characteristic. *Milk* undergoes no change. In *glucose broth* a uniform turbidity is produced and a film forms on the surface. There is no gas formation, but the medium becomes acid. In peptone water *indol* is produced.

In 0.25% potassium nitrate broth there is diffuse turbidity with film formation on the surface. The characteristic foaming due to gas formation occurs. The same appearances are found in 0.25% sodium nitrite broth.

This bacillus does not grow under anaërobic conditions in ordinary broth, but if 0.25% potassium nitrate be added growth and gas formation occur as before.

A culture was made in 0.25% potassium nitrate broth in a large flask which was filled quite full and tightly stoppered. From this flask a capillary glass tube was led off and the gas produced was collected over mercury. Samples of this gas were kindly analysed by Prof. Letts with the following result:

First 20 c.c.		Second 20 c.c.	
Nitrogen	99 %	Nitrogen	98 %
Carbonic acid	1 %	Carbonic acid	2 %

This bacillus therefore decomposes nitrate with the formation of free nitrogen.

The only other bacillus belonging to this group which was isolated was *B. pyocyaneus*. This produced the typical foam formation in

0.25 % potassium nitrate broth, but the gas produced was not analysed. Its other characters need not here be described.

B. Bacteria which cause the disappearance of the nitrate reaction, but do not produce gas in 0.25% potassium nitrate broth: B. hyponitrosus group.

Bacillus No. 2. This is a slender, slightly curved bacillus. In broth culture it forms short filaments which show a sluggish serpentine movement. It does not form spores, and it retains the stain in Gram's method. It liquefies gelatin slowly and irregularly; thus a gelatin slope culture shows a white layer along the track of the needle with deep pits produced by liquefaction especially at the lower part of the stroke.

Gelatin plate culture: The colonies appear after 2 days at 22° C. At first there is no sign of liquefaction, the surface colonies forming round white discs about 2 mm. in diameter, and the deep colonies uncharacteristic small spheres. After about 4 days liquefaction begins and proceeds rapidly around all the colonies.

Agar stroke culture is uncharacteristic, somewhat resembling that of *B. coli*.

Milk is coagulated in 7 days at 37° C. There is marked production of *indol* in peptone water. In glucose broth this bacillus does not grow readily. In a fermentation tube containing glucose broth diffuse growth occurs in the open limb of the tube, but none in the closed limb; some acid is produced but no gas.

In 0.25 % potassium nitrate broth a copious turbid growth occurs. No gas is produced, but the nitrate reaction is largely diminished in amount. In dilute nitrate broth the nitrate reaction disappears in 2 days at 37° C.

This bacillus was not identified and its chemistry was not further studied. Its action on nitrates was not so energetic as that of the next bacillus (No. 3) and it appeared to be better to choose the latter as an example of the group for further study from a chemical point of view.

Bacillus No. 3. This bacillus was isolated from 1 c.c. of the sprinkler effluent of Oct. 12th, 1905. For reasons which will appear later I have given it the name *B. hyponitrosus*. In form it is very short, almost coccus-like. It is actively motile and does not form spores. It does not retain the stain in Gram's method.

Gelatin plate culture: The colonies become visible after two days

at 22° C., as clear transparent round dew-drop like spheres. Under a low power of the microscope the *deep colonies* are perfectly round with a smooth contour and appear dark and granular. The *surface colonies* are larger and show a transparent wavy spreading margin, while the centre is dark and granular, the granulations becoming less marked towards the periphery. Usually a small dark round nucleus is seen of the same appearance as the deep colonies. This is often eccentrically situated and varies in size, apparently according to the depth in the gelatin from which the colony has grown to the surface. The surface colonies never exceed about 2 mm. in diameter. No liquefaction of the gelatin occurs.

Gelatin stroke culture: The growth is not unlike that of *B. coli*, but is rather harder and more adherent.

The growth on *agar* is soft, white, and not characteristic.

Milk is unchanged after 10 days at 37° C. No *indole* is formed in peptone water. In glucose broth growth does not readily take place. No growth takes place in ordinary broth under anaërobic conditions. If however nitrate be present, growth does occur. Even when exposed to air the growth in nitrate broth is more copious than that in ordinary broth.

In 0.25 % potassium nitrate broth the nitrate reaction disappears in from one to two days at 37° C., but no gas is produced.

Even in broth containing 1 % potassium nitrate growth takes place. In this strength of nitrate the growth at first takes the form of discrete colonies in the shape of small white spheres attached to the sides of the flask, or suspended freely in the liquid, the whole forming a very characteristic picture. Later diffuse growth occurs with uniform turbidity, and the nitrate reaction disappears in seven days at 37° C. Usually no gas is produced but I have occasionally observed some effervescence in cultures containing so much as 1 % nitrate.

This bacillus has now been grown on the ordinary laboratory media for a period of two and a half years since its original isolation. It grows readily at room temperature and does not require transplantation oftener than once a month. It has not changed its characters in any respect during this period. In particular its action on nitrates is as energetic as ever.

(4) *The Chemistry of B. hyponitrosus.*

As it appeared to be of considerable scientific interest, and of some practical importance with regard to the Belfast sewage problem, to

determine what becomes of the nitric nitrogen not appearing as gas, this question was fully investigated in connection with *Bacillus* No. 3. When this bacillus is grown in 1% nitrate broth until the nitrate reaction has disappeared, the culture becomes strongly alkaline, and if a tube containing it be boiled, enough ammonia is given off to turn moist litmus paper blue. This at once suggested that the nitrate was reduced to ammonia, but as it was found that ammonia was also produced, although not in such large amount, in ordinary broth cultures of the bacillus, it became necessary to estimate accurately the amount of ammonia produced in each case.

Cultures were made in flasks containing 100 c.c. peptone water (peptone 10 gm., sodium chloride 5 gm. to 1 litre) to which varying quantities of potassium nitrate, accurately weighed, were added. The flasks after inoculation with the bacillus were incubated at 37° C., and any ammonia given off in the incubator was collected and estimated by connecting the flask with a test tube containing 10 c.c. deci-normal sulphuric acid. After incubation the culture was made up to 100 c.c. and 20 c.c. were taken for the estimation of ammonia. This was done by distillation with magnesia, *in vacuo*, at a temperature of from 35° to 40° C., according to the method described by Nencki and Zaleski (1895). The ammonia given off was received in a definite quantity of deci-normal sulphuric acid, which was afterwards titrated against deci-normal caustic soda, methyl orange being used as indicator.

In another sample of the culture the total alkalinity was estimated by titration against deci-normal sulphuric acid, with methyl orange as indicator; by deducting the amount of ammonia obtained by distillation, an estimate of the amount of "fixed alkali" present was obtained.

Estimations of the total unoxidised nitrogen by the Kjeldahl method were also made in some cases.

Exp. 1. This was a control experiment with sterile peptone water containing 0.25% potassium nitrate.

20 c.c. on distillation with magnesia gave off ammonia requiring for its neutralisation 0.6 c.c. N/10 acid.

20 c.c. required to render it neutral to methyl orange 2.1 c.c. N/10 acid.

Therefore in 100 c.c.

NH_3 by distillation = $0.6 \times 5 = 3.0$ c.c. N/10 acid = 5.1 mgm.

Total alkali 10.5 c.c. N/10 „

Alkali not ammonia 7.5 c.c. N/10 „

Exp. 2. Culture in 100 c.c. peptone water, without nitrate, incubated for 48 hours at 37° C.

In 100 c.c.

NH ₃ in incubator required	0.5 c.c. N/10 acid	
NH ₃ by distillation = 1.4×5	= 7.0 c.c. N/10	„
Total ammonia	7.5 c.c. N/10	„ = 12.75 mgm.

Exp. 3. Culture in 100 c.c. peptone water containing 0.254 gm. potassium nitrate; 48 hours at 37° C. A trace of nitrite was still present

In 100 c.c.

NH ₃ in incubator required	0.3 c.c. N/10 acid	
NH ₃ by distillation = 2.7×5	= 13.5 c.c. N/10	„
Total ammonia	13.8 c.c. N/10	„ = 23.46 mgm.
Total alkali	39.5 c.c. N/10	„
Fixed alkali	26.0 c.c. N/10	„

Exp. 4. Culture in 100 c.c. peptone water containing 0.56 gm. potassium nitrate; 48 hours at 37° C. Nitrate still present in considerable amount; a trace of nitrite.

In 100 c.c.

NH ₃ in incubator required	0.3 c.c. N/10 acid	
NH ₃ by distillation = 2.8×5	= 14.0 c.c. N/10	„
Total ammonia	14.3 c.c. N/10	„ = 24.31 mgm.
Total alkali	36.5 c.c. N/10	„
Fixed alkali	22.5 c.c. N/10	„

In neither of the last two experiments had the nitrate reaction completely disappeared. They were therefore repeated, the cultures being allowed to remain in the incubator until the nitrate reaction was negative.

Exp. 5. Culture in 100 c.c. peptone water containing 0.25 gm. potassium nitrate; 3 days at 37° C. Nitrate reaction negative.

In 100 c.c.

NH ₃ in incubator required	0.8 c.c. N/10 acid	
NH ₃ by distillation = 3.2×5	= 16.0 c.c. N/10	„
Total ammonia	16.8 c.c. N/10	„ = 28.56 mgm.
Total alkali	41.0 c.c. N/10	„
Fixed alkali	25.0 c.c. N/10	„

Exp. 6. Culture in 100 c.c. peptone water containing 0.50 gm. potassium nitrate; 5 days at 37° C. Nitrate reaction negative.

In 100 c.c.

NH ₃ in incubator required	3.0 c.c. N/10 acid	
NH ₃ by distillation = 5.3×5	= 26.5 c.c. N/10	„
Total ammonia	29.5 c.c. N/10	„ = 50.15 mgm.
Total alkali	70.0 c.c. N/10	„
Fixed alkali	43.5 c.c. N/10	„

It is now desired to ascertain whether the increasing alkalinity of the culture exerted a retarding influence on the growth of the bacillus. This was done by adding to the culture at intervals, measured quantities of N acid in order to neutralise some of the alkali produced.

Exp. 7. Culture in 100 c.c. peptone water containing 0.52 gm. potassium nitrate; 5 days at 37° C. Nitrate reaction negative.

After 2 days 2.5 c.c. N sulphuric acid was added. On the next day the reaction was still strongly alkaline, and an additional 1.5 c.c. N acid was added. After 4 days nitrate was still present and the reaction was alkaline. In all 4.0 c.c. N acid was added, and 5 c.c. of the culture was withdrawn to test the alkalinity and the presence of nitrate. A correction for this had therefore to be made.

The total nitrogen was also estimated in this case by the Kjeldahl method.

In 100 c.c.

NH ₃ in incubator required	0.3 c.c. N/10 acid	
NH ₃ by distillation = 5.6×5 (20/19)	= 29.5 c.c. N/10	„
Total ammonia	29.8 c.c. N/10	„ = 50.6 mgm.
Total alkalinity	53.0 c.c. N/10	„
Fixed alkali	23.5 c.c. N/10	„
Add for 4 c.c. N acid	40.0 c.c. N/10	„
Total fixed alkali	63.5 c.c. N/10	„
Total nitrogen (Kjeldahl) as NH ₃		= 170.5 mgm.

From the last two experiments we obtain the remarkable result that an alkalinity amounting to 70% of a deci-normal solution (Exp. 6) does not interfere with the growth and vital activity of this bacillus. The amount of ammonia produced is practically identical, and the nitrate reaction disappeared in exactly the same time in both cases.

In the next experiment a still larger amount of nitrate was added; the culture was incubated until the nitrate reaction had disappeared, and the Kjeldahl nitrogen was again estimated.

Exp. 8. Culture in 100 c.c. peptone water containing 1.39 gm. potassium nitrate ; 9 days at 37° C. Nitrate reaction negative.

In 100 c.c.

NH ₃ in incubator required	2.0 c.c. N/10 acid	
NH ₃ by distillation = 7.3×5	= 36.5 c.c. N/10	„
Total ammonia	38.5 c.c. N/10	„ = 65.45 mgm.
Total alkali	114.0 c.c. N/10	„
Fixed alkali	78.0 c.c. N/10	„
Total nitrogen (Kjeldahl) as NH ₃		= 163.2 mgm.

Here the alkalinity of the culture is greater than that of a decinormal solution. The amount of ammonia is only slightly greater than in the previous experiment, and the Kjeldahl nitrogen, which of course includes the ammonia, is rather less.

Exp. 9. An estimation of the total nitrogen in sterile 1 % peptone water was now made by the Kjeldahl method.

After incineration and distillation the ammonia given off from 10 c.c. required for its neutralisation

in the first sample	9.9 c.c. N/10 acid
in the second sample	10.3 c.c. „
Average	<u>10.1 c.c.</u> „

In 100 c.c. therefore

Total nitrogen (Kjeldahl) as NH₃ = 171.7 mgm.

This is rather greater than the amounts obtained in Exps. (7) and (8) where amounts of nitrate equivalent respectively to 88 mgm. and 236 mgm. of ammonia had disappeared.

These results show clearly that the ammonia produced in the cultures is derived, not from the nitrate, but from the peptone.

We may now tabulate in a different form some of the results already obtained.

Deducting the amount of ammonia (5.1 mgm.) in the sterile peptone water from that in the cultures, we find the following figures for the amounts of ammonia produced.

Exp. No.	Days	Nitrate	NH ₃ produced
(2)	2	nil	7.65 mgm.
(3)	2	0.25 %	18.36
(4)	2	0.56	19.21
(5)	3	0.25	23.46
(6)	5	0.50	45.05
(7)	5	0.52	45.50
(8)	9	1.39	60.35

The presence of nitrate undoubtedly causes an increase in the formation of ammonia. This must be accounted for by the fact that it favours the growth of the bacillus enabling it to break down the peptone more rapidly. The amount of ammonia produced is however much more nearly proportionate to the age of the culture than to the amount of nitrate present.

If similarly we deduct the amount of "fixed alkali" found in the control experiment (7.5 c.c. N/10) from that found in the cultures, and suppose for the present that the remainder is due to KOH derived from KNO_3 , it is easy to calculate what percentage of the nitrate present is represented by the fixed alkali produced.

Exp. No.	Fixed alkali	KNO_3	Percentage of total
			KNO_3
(3)	18.5 c.c. N/10	187 mgm.	73.6 %
(4)	15.0 c.c.	152	25.0
(5)	17.5 c.c.	177	71.0
(6)	36.0 c.c.	364	72.9
(7)	56.0 c.c.	565	108
(8)	70.5 c.c.	713	51.1

We have seen that the nitrate disappearing is not represented by ammonia, nor by any other form of nitrogen which appears in a Kjeldahl analysis. If it disappeared in gaseous form, either as free nitrogen or as lower oxides, the whole of the potassium nitrate disappearing would necessarily be represented by fixed alkali in the culture. That this is not so, except in one case (Exp. 7), is clear from the above table. The fixed alkali accounts only for, at most, 73 % of the potassium nitrate.

This suggests, as the true explanation of the facts, that the nitrate is reduced to *hyponitrite*.

Divers (1899) has shown that when potassium amalgam is made to act on potassium nitrate, among other products of reduction, potassium hyponitrite (KNO) is formed. The hyponitrites of sodium and potassium are, like the carbonates, salts with an alkaline reaction. They are rather unstable, and on heating, with or without acid, they give off nitrous oxide gas (N_2O). They do not give the brucin sulphuric acid reaction, and they do not appear in a Kjeldahl analysis as they are at once decomposed on heating with sulphuric acid.

The "fixed alkali" therefore, in the above experiments, represents not a portion of the KNO_3 as KOH, but the whole of it as KNO . In the exceptional experiment (No. 7), sufficient sulphuric acid had been added to decompose most of the hyponitrite with formation of K_2SO_4 ,

and the alkali in combination with sulphuric acid is reckoned in the total fixed alkali produced.

That bacillus No. 3 reduces nitrate to hyponitrite and no further, and may therefore appropriately be named *B. hyponitrosus*, is further shown by analyses of the gas given off from cultures in nitrate peptone water when these are boiled with acid. It is possible to recover in this way the whole of the nitrogen disappearing as nitrate, in the form of nitrous oxide gas.

The gas analyses were carried out by means of the apparatus described by Haldane (1898), in which there is an automatic adjustment for changes of temperature and pressure during a series of observations.

Exp. 10. A large flask of 0.5% nitrate peptone water was inoculated with the bacillus and incubated at 37° C. until the nitrate reaction had disappeared. Sulphuric acid was then added, the flask gently heated over a Bunsen burner, and the gas given off collected over mercury. The first samples tested contained a considerable amount of carbonic acid as was shown by shaking up with lime water. Another sample of the gas collected in a test tube, after absorption of carbonic acid by means of moistened solid caustic soda, caused a burning splinter of wood to glow more brightly. This is evidence of the presence of nitrous oxide, as oxygen is practically out of the question. A small quantity of a strong solution of caustic soda was now introduced into a burette over mercury, the flask was heated gradually almost to boiling point, and the gas not absorbed by the caustic soda was collected and analysed.

A sample introduced into Haldane's apparatus measured 7.15 c.c. An approximately equal volume of hydrogen from a Kipp apparatus was introduced, the reading being now 14.66 c.c. The mixture was now ignited in an explosion pipette; an explosion took place, accompanied by a reduction in volume to 8.65 c.c. The amount of hydrogen used was therefore 6.01 c.c. and this is equivalent to the amount of nitrous oxide in the sample of 7.15 c.c., that is to say, 84% of the whole.

Another sample of the gas was introduced and passed over into the pyrogallic bulb. Some absorption took place. The remainder formed an explosive mixture with hydrogen as before and contained 64.5% of nitrous oxide. By this means the possibility of the explosion being due to oxygen is excluded, but on account of the great solubility of nitrous oxide it is impossible to obtain an accurate analysis if the gas is subjected to absorption before explosion.

In the next experiment the amount of nitrous oxide obtained from a definite quantity of nitrate was measured, and the amount of nitrous oxide given off on boiling the culture without the addition of acid was also ascertained. In each case the gas was boiled off under slightly reduced pressure by means of a special apparatus consisting of a large pipette connected to a mercury reservoir and surrounded by a steam jacket. From this apparatus it was easy to collect the gas in a nitrometer over mercury without any possibility of contamination with air. The gas collected was measured in the nitrometer, the temperature and barometric pressure being noted. Samples were then transferred to Haldane's apparatus for analysis. Enough residual nitrogen was left in the gas-analysis apparatus to prevent explosion and the combination with hydrogen was effected by means of the combustion pipette. This contains a spiral of platinum wire which can be brought to a bright red heat by means of a storage battery. After combustion the carbonic acid was estimated by absorption in the potash bulb.

Exp. 11. A flask of peptone water to which exactly 0.50 gm. potassium nitrate had been added was inoculated with *B. hyponitrosus* and incubated at 37° C. for 3 days. The nitrate reaction was then negative.

The whole culture measured exactly 225 c.c. Of this definite fractions were taken for analysis as follows.

From 60 c.c. of the culture, on boiling with acid, there was obtained

21 c.c. of gas at 21° C. and 758 mm.

The analysis of this gas gave:

	First sample	Second sample	Average
Nitrous oxide	90.7 %	91.4 %	91.0 %
Carbonic acid	6.5	5.5	6.0
Nitrogen	2.8	3.1	3.0

Thus from $60/225 \times 0.5$ gm. KNO_3 we obtain

$21 \times 91/100$ c.c. N_2O at 21° C. and 758 mm.,

therefore 1 gm. KNO_3 would yield

143.3 c.c. N_2O at 21° C. and 758 mm.

or (correcting for temperature and pressure)

133 c.c. N_2O at 0° C. and 760 mm.,

and from 1 gm. KNO_3 , 135 c.c. N_2O " "

is the theoretical amount.

As a control the gas from another sample of the culture was analysed by absorption in the potash and pyrogallic bulbs without combustion. This gave the following:

Nitrous oxide + carbonic acid	98 %
Nitrogen	2 %

This again excludes the presence of oxygen. The nitrous oxide is not so readily absorbed by the potash as is carbonic acid, but by continuing the absorption until a constant reading was obtained, 98 % of the whole sample disappeared, and no further absorption occurred in the pyrogallic bulb.

From 35 c.c. of the culture, on boiling without acid, there was obtained

7 c.c. of gas at 20° C. and 762 mm.

The analysis of this gas gave:

Nitrous oxide	81.5 %
Carbonic acid	7.6
Nitrogen	10.9

Thus from $35/225 \times 0.5$ gm. KNO_3 we obtain

$7 \times 81.5/100$ c.c. N_2O at 20° C. and 762 mm.

therefore 1 gm. KNO_3 would yield

73.4 c.c. N_2O at 20° C. and 762 mm.

or

68 c.c. N_2O at 0° C. and 760 mm.

Thus we obtain as nitrous oxide the following percentages of the total nitrate present,

on boiling the culture with acid	98.5 %
on boiling the culture without acid	50.3 %

This difference shows that the nitrous oxide is not present merely in solution, but that it is in combination as hyponitrite, which is decomposed more easily in acid than in alkaline solution on boiling.

Thus we find that in cultures of *B. hyponitrosus* in nitrate peptone water, the whole of the nitrate is reduced to hyponitrite, and remains in solution in this form, at least where the total amount of nitrate originally present does not exceed 0.25 %.

As has already been mentioned, in some cases gas formation does occur in cultures containing 1 % potassium nitrate. This is not a constant occurrence, and it was not found possible to collect the gas in sufficient quantities for a satisfactory analysis, but on one occasion I was able to detect in it the presence of nitrous oxide. The reason for

the difficulty in obtaining a sufficient quantity of the gas for analysis appears to be that the strictly anaërobic conditions necessary for the collection of the gas are not favourable to the growth of the bacillus. Under these conditions, in 1% nitrate peptone water, the growth only proceeds to the stage of discrete colonies; no diffuse growth occurs and only a small amount of gas is given off.

(5) *The relation of Oxygen to the growth of the Denitrifying Bacteria.*

The denitrifying bacteria are essentially aërobic, they can however grow under anaërobic conditions in the presence of nitrate, the nitrate supplying the necessary oxygen for their vital activities. On the other hand, under conditions of specially good aëration, these bacteria grow readily enough but they do not attack nitrate, the necessary oxygen being otherwise supplied.

Experiments on these points were made both with pure cultures of Bacilli Nos. 1, 2 and 3, and with sewage samples.

Cultures of the three bacilli were made in test tubes containing 0.25% nitrate broth and in the same broth without the addition of nitrate. One set of the cultures were placed under anaërobic conditions in Buchner's tubes. After incubation at 37° C. the anaërobic cultures in ordinary broth showed no growth. The order of the amount of growth in the other cases was as follows:

(1) Aërobic nitrate, (2) Aërobic, (3) Anaërobic nitrate.

In the nitrate cultures denitrification proceeded at the same rate under aërobic and anaërobic conditions.

The next experiments were done with a view to determine whether denitrification takes place under conditions of specially good aëration.

(1) Cultures were made in dilute peptone broth (1 in 20) containing 0.03% potassium nitrate, in small Erlenmeyer flasks, each flask receiving only 10 c.c. of the broth so that the liquid was spread out in a thin layer on the bottom of the flask and freely exposed to air.

In the case of Bacilli Nos. 1 and 2, the nitrate reaction was still present after 3 days at 37° C. In the case of Bacillus No. 3, the nitrate reaction had disappeared in 3 days; it was not tested earlier.

In ordinary test tube cultures containing 10 c.c. of this dilute nitrate broth the nitrate reaction in the case of all three bacilli disappears in 2 days at 37° C.

(2) Cultures of Bacillus No. 3 were made in 0.25% potassium

nitrate broth, (a) in test tubes containing 10 c.c., and (b) in Erlenmeyer flasks containing 10 c.c.

After 2 days at 37° C. the nitrate reaction had disappeared in the test tube, but was still present in the flask.

(3) Cultures of *Bacillus* No. 2 were made in 0.25 % potassium nitrate broth, (a) in test tubes containing 10 c.c., and (b) in Erlenmeyer flasks containing 10 c.c.

The cultures were incubated for 3 days at 37° C. and then allowed to stand for 2 days at room temperature.

Each culture was then made up to 50 c.c. with water, and the nitrite present was estimated by Griess' method. In another sample the nitrite was got rid of by heating in acid solution with urea, and the absence of nitrate in both cases demonstrated by the brucin method.

The percentage of the original nitrate remaining as nitrite was

(a) in test tube 9.3 %,

(b) in flask 33.9 %.

(4) Samples of septic tank and sprinkler effluents were mixed in equal quantities and allowed to stand in a stoppered bottle three quarters full. After 24 hours at room temperature the nitrate reaction had disappeared.

(5) 30 c.c. each of the same septic tank and sprinkler effluents were put in a large Erlenmeyer flask so as to form a layer about 1/8 inch in depth and expose a large surface in contact with air. After 4 days at room temperature the nitrate reaction was still present.

These results show that denitrification does not proceed so rapidly either in pure cultures of the bacteria, or in the sewage, when there is free exposure to air.

(6) *Estimation of the number of Denitrifying Bacteria in the various samples of Sewage.*

Dilutions of the sewage samples were made as before, and two distinct methods were used for demonstrating the presence of denitrifying bacteria.

First method. 1 c.c. of each dilution tested was added to a tube filled to 10 c.c. with dilute nitrate peptone water (0.02 % potassium nitrate); the tubes were incubated for 3 to 4 days and then tested for the nitrate reaction. The disappearance of this reaction was taken as evidence of the presence of denitrifying bacteria.

Second method. Durham's fermentation tubes containing 0.25 %

nitrate broth were used, and the appearance of well marked gas formation (at least 1/10 of the closed limb) was regarded as evidence of the presence of typical denitrifying bacteria belonging to the class represented by *B. Stutzeri*.

In using nitrate containing media it was kept in mind that there is a class of bacteria represented by *B. denitrificans*, which decompose nitrite with formation of free nitrogen, but which do not attack nitrate. As however many of the bacteria present in sewage can reduce nitrate to nitrite, it seemed better to use the nitrate media, and to interpret the results as indicating the presence of either (1) bacteria capable of destroying nitrate, or (2) a combination of bacteria which can reduce nitrate to nitrite with nitrate destroying organisms.

On Dec. 12th, the samples were examined by both the methods mentioned above, and the detailed observations for that date are given below as an example of the results obtained.

Sample	Dilutions				
	1/10	1/100	1/1000	1/10,000	1/100,000
<i>First method :</i>					
S. T. effluent	—	+	+	+	no growth.
Sp. effluent	+	+	0	0	„
Upper Denitrifying	—	+	+	0	„
Lower Denitrifying	—	+	+	0	„
<i>Second method (gas formation) :</i>					
S. T. effluent	—	+	+	0	0
Sp. effluent	0	0	0	no growth	
Upper Denitrifying	—	+	0	0	0
Lower Denitrifying	—	0	0	0	no growth.

In the above table

+ indicates disappearance of nitrate reaction, or gas formation.

0 indicates positive nitrate reaction, or no gas formation, although growth occurred.

— indicates “not tested.”

In these samples denitrifying bacteria which do not produce gas are obviously more numerous than the typical denitrifiers. Thus with 1/10 c.c. sprinkler effluent, and 1/100 c.c. effluent from the lower “denitrifying” bed, no gas formation occurred although the nitrate reaction disappeared with 1/100 and 1/1000 c.c. respectively. In this case larger quantities were not tested, but it is to be noted that if a sufficient quantity of any of the sewage samples were inoculated into 0.25 % nitrate broth, gas formation always occurred. This suggests

that under natural conditions all the nitrate disappears as free nitrogen. We know that the formation of nitrite is a constant stage in the reduction of nitrate both by the typical denitrifiers and by those belonging to the *B. hyponitrosus* group. It is probable that in a similar way hyponitrite also represents a stage in the reduction of nitrate by the typical denitrifiers, and that in the presence of both classes of bacteria, the reduction proceeds further, with formation of free nitrogen. This is a point which requires further investigation.

For comparison with the numbers of denitrifying bacteria, enumerations were also made of *B. coli* and streptococci, and also of the total bacteria growing at 22° and 37° C.

The results of the enumerations on the various dates are given in Table VI, and also the averages for the whole series. It will be observed that the figures agree very closely with the counts made in February (Table III).

A very large reduction of bacteria in the sprinkler effluent is again seen and this is more marked in the denitrifiers than in any other group. *B. coli* is about four times as numerous as the denitrifying bacteria, and of these, as we have already seen, the class which does not produce gas is the more frequent. The great reduction in the denitrifying bacteria in the sprinkler effluent is further evidence that denitrification does not readily occur where there is very free aëration.

But the fact that denitrifying organisms do occur in considerable numbers in the sprinkler effluent calls for remark. When the sprinkler effluent is incubated it does not lose its nitrate (Exp. 2, p. 625) although denitrifying bacteria are present in it. This can only be because it does not contain enough organic material to serve for their growth. It was in fact found that *Bacillus* No. 2 could not grow in sprinkler effluent which had been filtered through porcelain. If however a small amount of nutrient broth were added, growth and denitrification took place.

(7) *Conclusions.*

We have seen in the foregoing that the combination of a percolating bed (the sprinkler), with contact beds in which denitrification occurs, gives rise to a final effluent very similar both in chemical and bacteriological characters to that obtained by double contact treatment.

It is probable therefore that in the contact beds a considerable proportion of the organic nitrogen disappears as gas as a result of alternate nitrification and denitrification. In the sprinkler the free exposure to air renders it unlikely that any appreciable amount of denitrification

occurs. We may therefore take the excess of nitrate in the sprinkler effluent over that present in the effluent from the contact beds as a rough indication of the amount of nitrogen disappearing as gas in double contact treatment. This amounts to about 50 % of the nitrogen disappearing from the contact bed effluent (Table IV).

We can now easily understand why a contact bed effluent as a rule shows less nitrate than the effluent from a percolating bed. The amount of nitrate present is a direct measure of the degree of aëration to which the sewage has been exposed. Equally good effluents as regards purification may be obtained by means of contact treatment, or from a continuously working percolating bed. In the former a large amount of nitrogen disappears as gas; in the latter it is represented by nitrate. If the nitrate disappears completely from a contact bed effluent it is of course a sign that the bed is being overworked, the denitrifying process has been allowed to proceed too far; in other words the bed has been filled too frequently or too long, and sufficient interval has not been allowed for aëration and the active processes which accompany it during the so-called "resting" stage.

It also becomes clear why a highly nitrated effluent is less liable to undergo objectionable putrefactive changes than one less rich in nitrates, but otherwise similar. The denitrifying bacteria utilise the oxygen of the nitrate in breaking down complex nitrogenous substances under what are practically aërobic conditions. Anaërobic putrefaction with its resulting objectionable products is thus, to some extent at least, prevented. It is only putting the matter in another way to say that the nitrate oxidises the organic matter present. That it does so is due to the activity of the denitrifying bacteria. When *B. hyponitrosus* is grown in peptone solution containing nitrate it breaks down the peptone into ammonia much more readily than in the absence of nitrate. In neither case does it produce any indole, a typical product of putrefaction. So long as any nitrate is present, the cultures of this bacillus have no putrefactive odour. When the nitrate disappears there is a slight, but not very offensive odour of putrefaction.

The practical result of this study of denitrification, in its application to the Belfast sewage problem, appears to be that it is impossible to get rid of all the nitrate in the effluent without materially lessening the purification effected. The amount of organic material in a good effluent like that from the sprinkler is not sufficient for the growth of the denitrifying bacteria, and as we have seen, the disappearance of nitrate from an effluent is a sign that the bed is being overworked.

It may indeed, with reason, be asked whether it is desirable, even under the special conditions obtaining in Belfast, to try to get rid of any of the nitrate.

It is not certain that the living green sea-weeds can assimilate any appreciable amount of nitrate; and it is not improbable, on the other hand, that the nitrate may be very valuable in preventing objectionable putrefactive changes in the dead sea-weed, in virtue of the activity of the denitrifying bacteria.

NITRIFICATION AND OTHER BACTERIAL PROCESSES.

That the rôle of bacteria in the purification of sewage is a somewhat limited one, which by no means justifies the application of such terms as "bacteria beds" and "bacterial purification," appears from what has already been said. There is however one other stage in the process which may with great probability be ascribed to bacterial action, the stage namely of nitrification.

The nitrifying bacteria, first isolated and studied by Winogradsky, are divided into two groups, viz.

- (1) *Nitrite bacteria* which convert ammonia into nitrite.
- (2) *Nitrate bacteria* which carry the oxidation a stage further, and convert nitrite into nitrate.

These organisms do not grow on the ordinary laboratory media used for the cultivation of other bacteria. Their growth, in pure culture at least, is inhibited by the presence of more than minimal amounts of organic material, the nitrite formers being in this respect more sensitive than the nitrate formers. On the other hand, the presence of ammonia in any appreciable quantity inhibits the growth of the nitrate bacteria. Hence, when ammoniacal solutions suitable for the growth of the nitrite bacteria, are inoculated with a mixture of the two kinds, the process of nitrification takes place in two distinct stages, no nitrate being formed until practically all the ammonia has been converted into nitrite.

In view of these facts it is difficult to correlate the nitrification which occurs in nature, and in particular that which occurs in sewage purification, with the activity of these bacteria. In nature the process proceeds rapidly to the stage of nitrate in the presence of a large amount of organic material, and in spite of the presence of a large amount of ammonia.

A reasonable explanation of this difficulty has however been given by Winogradsky (1904).

This depends in the first place upon observations made by Omelianski (1899), who has shown that in combination with *B. ramosus*, a common soil bacterium of the "subtilis" group, the nitrite former can grow and cause the oxidation of ammonia, even in dilute peptone broth. When, under certain conditions, *B. ramosus* and the nitrite former are grown together in dilute peptone broth (1 in 20) ammonia is first produced and after 7 days nitrite appears. When in addition to these two bacteria the nitrate former is inoculated in the dilute broth, nitrate is formed, but only after about a month. It appears therefore that under conditions such as occur in nature the growth and activity of the nitrite forming bacteria are not interfered with by the presence of organic material.

The further difficulty of the formation of nitrate in the presence of ammonia is explained by the results of certain experiments of Boullanger and Massol (1903). These observers showed that when an ammoniacal solution, inoculated with both forms of the nitrifying bacteria, was allowed to undergo complete oxidation in contact with clinker and then withdrawn, a further supply of the ammoniacal solution poured on to the same clinker did not then interfere with the activity of the nitrate formers, but the oxidation proceeded at once as far as nitrate and only traces of nitrite could be found. In this case a growth of the nitrate forming bacteria had been established on the surface of the clinker, in a manner which exactly corresponds to what takes place in the "ripening" of a bed in sewage purification; and it appears that ammonia has a much greater power of inhibiting the growth of the nitrate forming bacteria than of interfering with their activity when growth has been once established.

In view of these two sets of observations, the conditions of which correspond to those present in the treatment of sewage in a percolating bed, there is good reason for ascribing the nitrification which occurs there to the activity of the nitrifying bacteria described by Winogradsky.

Before we can rightly estimate the relative importance of bacteria and of the higher forms of life in the chemical changes which lead to the regeneration of the adsorptive capacity of a bed much more work requires to be done. In particular the fauna and flora of an efficiently working bed should be studied and contrasted with those of a bed which is being overworked or is in the condition known to engineers as "sick."

There can be little doubt that bacteria play an important part in breaking down the large proteid molecule of those colloid substances

which are readily "adsorbed"; the simpler substances thus produced are easily oxidised to soluble inorganic compounds which are no longer retained by adsorption but pass out into the effluent. How far this oxidation is a purely chemical process and how far it is dependent on the activity of vegetable and animal forms of life, is a matter which requires further investigation. In the case of ammonia the oxidation to nitrate is, as we have seen, almost certainly dependent on bacterial action. On the other hand the great destruction of bacteria which takes place in the beds can be most readily accounted for by the activity of higher forms of life. It is however possible that the presence of adsorbed or condensed oxygen, while it favours the growth of the nitrifying micro-organisms, is unfavourable to the existence of the ordinary bacteria.

The work described in this paper was carried out partly in the Pathological Laboratory of Queen's College, Belfast, and partly in the Pathological Laboratory of the University of Manchester. Part of the work on denitrification was done jointly with Dr T. Carnwath.

I desire here to express my thanks to Prof. Letts and Prof. Milroy for their kind assistance in the chemical part of the work, and to Prof. Lorrain Smith and Dr C. Powell White for valuable suggestions at various stages of the investigation.

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TABLE I.

*Detailed Results of First Series.**Crude Sewage.*

Date	Bacteria per c.c.			<i>B. coli</i> present in fraction of 1 c.c. (or number per c.c.)	<i>Streptococci</i>	<i>B. enteritidis</i>
	Gelatin at 22° C.	Agar at 37° C.	Spores			
July 22, '04	16,000,000	—	7,500	1/100,000	—	1/1000
„ 26	90,000,000	—	500	(160,000)	—	1/100
Aug. 5	9,000,000	4,500,000	470	1/100,000	1/100,000	1/100
„ 12	40,000,000	3,300,000	1,700	1/100,000	(less than 100,000)	(less than 10)
„ 23	16,300,000	1,500,000	490	1/100,000	1/1000,000	1 c.c.
Average	34,000,000	3,100,000	2,130			

Screened and Settled.

July 22	17,000,000	—	5,500	1/100,000	1/100,000	1/1000
„ 26	20,000,000	—	700	(220,000)	—	1/100
Aug. 5	11,000,000	2,300,000	220	1/100,000	1/100,000	1/1000
„ 12	23,000,000	2,300,000	530	1/1000,000	(less than 100,000)	(less than 10)
„ 23	15,000,000	700,000	240	1/100,000	1/1000,000	1 c.c.
Average	17,200,000	1,760,000	1,440			

Septic Tank.

July 22	10,000,000	—	(less than 1,000)	1/100,000	—	1/100
„ 26	4,000,000	—	530	(60,000)	—	1/100
Aug. 5	6,000,000	1,200,000	160	1/1000,000	(less than 100,000)	1/100
„ 12	10,000,000	450,000	690	1/100,000	(less than 100,000)	(less than 10)
„ 23	12,000,000	560,000	320	1/1000,000	1/100,000	1 c.c.
Average	8,400,000	736,000	540			

Lower A. Bed Effluent.

July 22	1,000,000	—	(less than 100)	(less than 100,000)	—	1/100
„ 26	1,700,000	—	—	(20,000)	—	1/100
Aug. 5	6,500,000	400,000	40	1/100,000	1/100,000	1/10
„ 12	1,400,000	(less than 100,000)	140	1/100,000	(less than 100,000)	1/100
„ 23	2,700,000	320,000	90	1/100,000	1/10,000	—
Average	2,660,000	270,000	90			

Lower B. Bed Effluent.

July 22	5,000,000	—	(less than 100)	1/100,000	—	1/100
„ 26	1,700,000	—	800	(45,000)	—	1/100
Aug. 5	4,500,000	1,100,000	150	1/100,000	1/100,000	1/10
„ 12	2,500,000	600,000	250	1/1000,000	(less than 100,000)	(less than 10)
„ 23	3,900,000	470,000	150	1/100,000	1/1000,000	—
Average	3,780,000	723,000	290			

Bacteriology of Sewage Purification

TABLE I. (Continued.)

Lower D. Bed Effluent.

Date	Bacteria per c.c.			<i>B. coli</i> present in fraction of 1 c.c. (or number per c.c.) (less than 100,000)	<i>Streptococci</i> present in fraction of 1 c.c. (or number per c.c.) (less than 100,000)	<i>B. enteritidis</i> (less than 10)
	Gelatin at 22° C.	Agar at 37° C.	Spores			
July 22	1,000,000	—	—	—	—	1/100
„ 26	3,000,000	—	500	1/100,000	—	1/10
Aug. 5	3,000,000	500,000	100	1/100,000	(less than 100,000)	1/100
„ 12	2,100,000	400,000	400	1/100,000	(less than 100,000)	(less than 10)
„ 23	5,200,000	750,000	150	1/1000,000	1/100,000	—
Average	2,850,000	550,000	290			

Lower F. Bed Effluent.

July 22	1,500,000	—	(2,100 ?)	—	—	1/100
„ 26	2,000,000	—	200	1/100,000	—	1/10
Aug. 5	3,000,000	900,000	210	(less than 100,000)	(less than 100,000)	1/10
„ 12	3,150,000	500,000	370	1/100,000	(less than 100,000)	(less than 10)
„ 23	3,800,000	470,000	170	1/1000,000	1/10,000	1 c.c.
Average	2,690,000	623,000	230			

Lower G. Bed Effluent.

July 22	1,000,000	—	—	1/100,000	—	1/20
„ 26	2,700,000	—	300	(50,000)	—	1/100
Aug. 5	5,000,000	850,000	90	(less than 100,000)	1/100,000	1/100
„ 12	2,500,000	350,000	290	(less than 100,000)	(less than 100,000)	(less than 10)
„ 23	3,300,000	410,000	130	1/100,000	1/10,000	1 c.c.
Average	2,900,000	536,000	200			

(Note:—The numbers given in brackets for *B. coli* on July 26th were obtained from counts on Drigalski-Conradi plates.)

Untreated Sewage.

(i.e. Crude Sewage, Screened and Settled, and Septic Tank).

	<i>B. coli</i> 15 examinations	<i>Streptococci</i> 10 examinations	<i>B. enteritidis</i> 15 examinations
In 1/10,000 c.c.	1 time	4 times	6 times in 1 c.c.
In 1/100,000 c.c.	11 times	4 „	6 times in 1/100 c.c.
In 1/1000,000 c.c.	3 „	2 „	3 times in 1/1000 c.c.
Average	886,000 per c.c.	813,000 per c.c.	800 per c.c.

Effluents from Contact Beds.

	<i>B. coli</i> 24 examinations	<i>Streptococci</i> 15 examinations	<i>B. enteritidis</i> 22 examinations
In 1/10,000 c.c.	8 times	10 times	6 times in 1 c.c.
In 1/100,000 c.c.	13 „	4 „	6 times in 1/10 c.c.
In 1/1000,000 c.c.	3 „	1 time	10 times in 1/100 c.c.
Average	607,000 per c.c.	332,000 per c.c.	161 per c.c.

Per cent. Reduction in Numbers in the Effluents.

<i>B. coli</i>	<i>Streptococci</i>	<i>B. enteritidis</i>
31.5 %	59.2 %	80 %

TABLE II.

Average of Five Examinations, First Series.

	Bacteria per c.c.			Spores
	On gelatin at 22° C.	On agar at 37° C.		
Crude Sewage	34,260,000	3,100,000		2130
Screened and settled	17,200,000	1,760,000		1440
Septic Tank	8,400,000	736,000		540
Bed A	2,660,000	273,000		90
Bed B	3,780,000	723,000		290
Bed D	2,850,000	550,000		290
Bed F	2,690,000	623,000		230
Bed G	2,900,000	536,000		200
(Aug. 23, Sprinkler	1,400,000	80,000		100)

	Per cent. reduction on crude sewage.			Per cent. reduction on crude sewage.			Per cent. reduction on septic tank.		
	Bacteria			Chemical results			Bacteria		
	Gelatin	Agar	Spores	Free NH ₃	Alb. NH ₃	Oxyg. abs. test	Gelatin	Agar	Spores
Screened Sewage	50·0	43·0	31·0	(gain) 12	25	23	—	—	—
Septic Tank	75·4	76·3	74·0	(gain) 40	45	38	—	—	—
Bed A	92·3	91·2	95·8	(loss) 85	74	83	68·3	63	83
Bed B	89·0	76·7	86·0	43	71	77	55·0	2	46
Bed D	92·0	82·3	86·0	56	73	81	66·0	26	46
Bed F	92·2	80·0	89·0	52	79	79	68·0	16	57
Bed G	91·6	83·0	90·0	37	73	73	65·5	28	63
(Aug. 23 Sprinkler	91·5	94·7	—	—	—	—	88·4	86	—)

(Note :—The bacteria growing on agar at 37° C. were only counted on three occasions, and the figures for the sprinkler are based on one examination.)

TABLE III.

Detailed Results of Second Series.

Date	Bacteria per c.c.		<i>B. coli</i> present in fraction	<i>Streptococci</i> of 1 c.c.
	Gelatin at 22° C.	Agar at 37° C.		
Jan. 25, '05	3,750,000	220,000	1/100,000	(1/1000,000)
Feb. 1	2,700,000	100,000	1/10,000	1/10,000
„ 8	4,500,000	450,000	1/100,000	1/10,000
„ 15	6,150,000	1,500,000	1/100,000	1/100,000
„ 22	350,000	210,000	1/10,000	1/1000
Mar. 1	2,300,000	170,000	1/100,000	1/10,000
Average	3,300,000	440,000	349,000	133,000 per c.c.
<i>Sprinkler Effluent.</i>				
Jan. 25	500,000	35,000	1/10,000	1/10,000
Feb. 1	250,000	5,000	(less than 10,000)	(less than 10,000 per c.c.)
„ 8	180,000	6,000	1/1000	1/10,000
„ 15	285,000	57,000	1/1000	1/1000
„ 22	5,000	3,000	(less than 1000)	(less than 1000 per c.c.)
Mar. 1	110,000	2,500	1/1000	(less than 1000 per c.c.)
Average	220,000	18,000	8,000	12,000 per c.c.
<i>Mixture of Septic Tank and Sprinkler Effluent (calculated).</i>				
Jan. 25	2,100,000	127,000	—	—
Feb. 1	1,500,000	52,000	—	—
„ 8	2,300,000	230,000	—	—
„ 15	3,200,000	780,000	—	—
„ 22	200,000	120,000	—	—
Mar. 1	1,400,000	120,000	—	—
Average	1,780,000	238,000	178,000	72,000 per c.c.
<i>Effluent from 1st Contact Bed (Upper denitrifying).</i>				
Jan. 25	950,000	45,000	1/10,000	1/10,000
Feb. 1	1,200,000	50,000	1/10,000	(less than 10,000 per c.c.)
„ 8	3,000,000	150,000	1/10,000	1/100,000
„ 15	2,900,000	890,000	1/10,000	1/10,000
„ 22	150,000	40,000	1/1000	1/10,000
Mar. 1	1,800,000	51,000	1/10,000	1/10,000
Average	1,670,000	204,000	42,000	79,000 per c.c.
<i>Effluent from 2nd Contact Bed (Lower denitrifying).</i>				
Feb. 22	125,000	30,000	1/1000	1/1000
Mar. 1	600,000	19,000	1/1000	1/1000
Average	360,000	25,000	(less than) 10,000	(less than) 10,000 per c.c.

TABLE IV.

Detailed Results of Second Series.

Chemical Examinations by Prof. Letts.

Septic Tank.

Date	Oxygen absorbed (4 hours at 27° C.)	Parts per 100,000			
		Nitrogen as			
		Free NH ₃	Albuminoid NH ₃	Nitrates	Nitrites
Jan. 25, '05	7.98	3.46	0.70	—	—
Feb. 1	6.24	3.30	0.74	—	—
„ 8	6.88	2.96	0.74	—	—
„ 15	6.88	3.62	0.82	—	—
„ 22	5.95	2.58	0.62	—	—
Mar. 1	5.33	3.29	0.47	—	—
Average	6.54	3.20	0.68	—	—

Sprinkler Effluent.

Jan. 25	2.68	1.48	0.39	1.32	—
Feb. 1	2.59	1.07	0.33	1.32	traces
„ 8	2.56	0.74	0.37	1.51	„
„ 15	2.56	1.43	0.41	1.40	0.12
„ 22	2.27	0.49	0.29	1.98	traces
Mar. 1	2.07	0.56	0.27	2.46	0.01
Average	2.45	0.96	0.33	1.66	—

Effluent from 1st Contact Bed (Upper denitrifying).

Jan. 25	3.05	1.48	0.37	(0.82)*	0.0
Feb. 1	2.96	1.40	0.37	0.00	0.0
„ 8	3.04	1.32	0.37	0.00	0.0
„ 15	3.04	1.48	0.45	0.00	0.0
„ 22	2.49	0.95	0.37	0.00	0.0
Mar. 1	2.27	0.93	0.27	0.25	0.0
Average	2.80	1.26	0.36	0.05	—

Effluent from 2nd Contact Bed (Lower denitrifying).

Feb. 22	1.95	0.58	0.25	0.12	traces
Mar. 1	1.74	0.82	0.21	0.33	—
Average	1.84	0.69	0.23	0.26	—

* The upper denitrifying bed had only recently been started; this figure is therefore not included for the average.

TABLE V.

Average of Six Examinations, Second Series.

				Denitrifying Beds	
				Upper	Lower
Percentage reduction in bacteria calculated on...	Septic Tank	...	Gelatin at 22° C.	93.4 %	50 %
			Agar at 37° C.	96.0	53
	Mixture of Septic Tank & Sprinkler		Gelatin at 22° C.	—	6
			Agar at 37° C.	—	14
Percentage chemical purification calculated on...	Septic Tank	...	Oxygen absorbed	63	57
			Free NH ₃	71.5	61
			Alb. NH ₃	51	48
	Mixture of Septic Tank & Sprinkler		Oxygen absorbed	—	37
			Free NH ₃	—	39
			Alb. NH ₃	—	28

(Note :—There were only two examinations of the effluent from the lower denitrifying bed.)

TABLE VI.

*Enumeration of Denitrifying Bacteria, etc.**Septic Tank.*

(Disappearance of "nitrate reaction" in 0.02% nitrate peptone water.)

Date	Bacteria per c.c.		<i>E. coli</i> present	<i>Streptococci</i> in fraction of 1 c.c.	Denitrifying bacteria
	Gelatin at 22° C.	Agar at 37° C.			
Oct. 4, '05	1,000,000	750,000	1/100,000	1/10,000	1/10,000
„ 12	1,000,000	140,000	1/100,000	1/100,000	1/10,000
„ 30	2,700,000	210,000	1/100,000	—	1/10,000
Dec. 12	—	—	1/10,000	1/10,000	1/10,000
(Gas formation in 0.25% nitrate broth.)					
Nov. 17	6,500,000	1,100,000	1/100,000	1/10,000	1/10,000
Dec. 12	—	—	—	—	1/1000
Average	2,800,000	550,000	410,000	162,000	100,000 per c.c.

Sprinkler Effluent.

(By "nitrate reaction" in 0.02% nitrate peptone water.)

	(less than 100,000)	(less than 10,000)	(less than 1000)		
Oct. 4				1/1000	1/100
„ 12	70,000	10,000	1/1000	1/10,000	1/10
„ 30	200,000	26,000	1/1000	—	1/1000
Dec. 12	—	—	1/1000	1/1000	1/1000

(By gas formation in 0.25% nitrate broth.)

Nov. 17	460,000	170,000	1/1000	1/1000	1/10
Dec. 12	—	—	—	—	(less than 10)
Average	195,000	52,000	4,000	16,000	1,700 per c.c.

Effluent from Upper Denitrifying Bed.

(By gas formation in 0.25% nitrate broth.)

Nov. 17	2,500,000	710,000	1/10,000	1/10,000	1/1000
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(By "nitrate reaction" in 0.02% nitrate peptone water 1/1000.)

Dec. 12	—	—	1/10,000	1/10,000	1/100
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Effluent from Lower Denitrifying Bed.

(By gas formation in 0.25% nitrate broth.)

Nov. 17	2,200,000	600,000	1/10,000	1/10,000	1/10,000
Dec. 12	—	—	1/10,000	1/1000	(less than 100)

(By "nitrate reaction" in 0.02% nitrate peptone water.)

Dec. 12	—	—	—	—	1/1000
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THE PRINCIPLES INVOLVED IN THE STANDARDISATION OF DISINFECTANTS AND THE INFLUENCE OF ORGANIC MATTER UPON GERMICIDAL VALUE.

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CHAPTER I. INTRODUCTION.

THE importance of the standardisation of disinfectants is universally admitted but no unanimity of opinion has been arrived at regarding the conditions under which germicidal value should be determined. It is the precise conditions under which the observations are to be made, upon which agreement is so necessary, but so difficult to obtain. An agreement is essential for the reason that the efficiency of every different class of disinfectant is differently affected by alteration in the circumstances of its use. Standard conditions for testing purposes have been difficult to define because in practice disinfectants are used as germicides under a variety of conditions, of which the following afford typical instances :—

(1) For the chemical sterilisation of surgical instruments and the hands of the operator, and for various antiseptic lotions and washes, in which case they are mixed with water and are not subjected to the possibly disadvantageous admixture of other substances.

(2) For the purpose of preserving sera, vaccines, etc. against possible bacterial contamination, in which case they act in the presence of a considerable amount of soluble organic matter.

(3) For the disinfection of utensils, soiled linen, closet pans, dejecta, etc. and for the washing of walls and articles of furniture.

By far the greatest quantity of disinfectants is sold for one or other of the purposes detailed under No. 3.

The determination in water of the germicidal value of a preparation will give a serviceable indication if it is to be used for some purposes, but will not necessarily be a reliable guide to its value when mixed, for example, with blood serum or dusty water or the dejecta of a typhoid patient.

It is impracticable to standardise disinfectants under a great variety of circumstances, but it is important to ascertain the effect upon various classes of disinfectants of such conditions as those under which they are commonly employed, and it is likely that the determination of the value of disinfectants in the presence of some definite amount of organic material, both particulate and in solution, will afford a more useful guide to their value from a hygienic point of view.

The work published during the last few years upon the nature of the disinfection process and the laws it obeys (Krönig and Paul (1897), Madsen and Nyman (1907), H. Chick (1908)) has made it possible to formulate the fundamental conditions which must be satisfied in the comparison of germicidal values. These conditions and means suggested for satisfying them are first dealt with in the present paper.

Experiments have been made with three types of disinfectants commonly employed, viz. phenol, mercuric chloride and two emulsified disinfectants¹, referred to as disinfectants "A" and "B." Each of these types has been studied with sporing organisms, *B. subtilis* and *B. anthracis*, with *B. typhosus* and *B. paratyphosus* and with *Staphylococcus aureus* and *B. pestis*. The influence of the virulence or otherwise of the strain of organism has also been considered.

Particular attention has been given to the influence of various forms of organic matter both in suspension and solution upon the germicidal action of these types of disinfectant, experiments being made upon some or all of the above organisms.

The results of our experiments have led us to suggest some modification in the methods commonly employed for the standardisation of disinfectants, and at the end of the paper we put forward a scheme for the standardisation of disinfectants intended for use upon dejecta, or for other purposes involving the presence of a considerable amount of suspended organic matter.

Historical. Systematic experiments comparing the values of different substances to hinder putrefaction were made as long ago as 1750 by *Pringle* (1750). *Buchholtz* (1875) made extensive experiments with a great variety of reputed disinfectants, using an infusion of tobacco leaves, and *Jalan de la Croix* (1881) covered much the same ground, using broth made from meat and the organisms naturally occurring in it. *Baxter* (1875) used vaccine lymph and glanders nodules as test materials, and emphasized the influence of associated organic matter in diminishing the value of the disinfectant.

Robert Koch (1881), however, was the first to compare the germicidal value of various disinfectants upon *pure* cultures of bacteria. He worked with emulsions of anthrax spores dried upon silk threads and estimated the time of survival in solutions of the then known disinfectants, with the result that mercuric chloride has since been justly regarded as the most efficient disinfectant where spores are concerned. Koch's thread method was also used by *Behring* (1890) in a number of

¹ The active principles in these emulsified disinfectants were derivatives of higher tar acids; the greater part of these acids distilled over between 210 and 300° C. Disinfectant A contained glue, and Disinfectant B resin and soft soap as emulsifiers. Both A and B formed extremely fine emulsions when diluted with distilled water.

experiments in which comparison was made of the germicidal value of a series of disinfectants upon anthrax spores; Behring also published a number of results of experiments made with many vegetative forms, e.g. anthrax bacilli, *B. cholerae*, *B. typhosus*, streptococci and staphylococci. Geppert (1889 and 1891) confirmed Koch's results, but criticised the thread method in view of the unavoidable carrying-over of traces of disinfectant into the test culture, these traces preventing growth of the organisms adherent to the thread, although they might not have lost their vitality at the moment of subculture. On adding to the test culture sufficient ammonium sulphide to precipitate the traces of mercuric chloride that were thus carried over, when working directly with emulsions of bacteria in place of the silk threads soaked in broth cultures, Geppert found that the organisms might still grow¹, and concluded that the germicidal value of mercuric chloride, though doubtless preeminent where spores are concerned, had been overestimated. Gruber (1891) advocated working with emulsions of bacteria and supported Geppert's view as regards the overcarriage of HgCl_2 , the misleading results of which he avoided by successive dilution of the test sample.

About the same time Creolin, the first of many emulsified disinfectants, received a great deal of attention from many workers; its germicidal value was compared with that of the older disinfectants, various organisms and different methods being used. Esmarch (1887) found it to be far superior to carbolic acid for the disinfection of emulsions of *B. cholerae* and streptococci, and less efficient for disinfection of anthrax spores, using the thread method. Henle (1889), also working with emulsions of bacteria, obtained excellent results with *B. typhosus*, while Fraenkel (1889), working with anthrax spores and the thread method, demonstrated the high disinfecting power of cresols and higher phenols, to which the germicidal value of creolin as well as that of crude carbolic acid is due.

Krönig and Paul (1897) devised a method for estimating germicidal value of disinfectants with anthrax spores, which had none of the drawbacks of Koch's thread method. Garnets were selected of similar size, carefully cleaned and dipped into an emulsion of sporing anthrax bacilli which was allowed to dry on their surface in a thin film. The garnets were then immersed in the solution of the disinfectant in question; from time to time a definite quantity was taken out, the disinfectant carried over was removed by gentle washing with water and, if necessary, with a precipitant (e.g. ammonium sulphide in the case of mercuric chloride). The garnets were then vigorously shaken in a measured quantity of water to detach the adherent spores and a constant amount of the washings was plated. The number of germinating organisms was counted, it having been shown that by vigorous shaking in water a fairly constant proportion of the total organisms was detached. By this method comparison could be made of the velocity of disinfection in the case of different disinfectants under different conditions. Krönig and Paul published a large number of very careful experiments, made with disinfectants of every class and in widely varying concentrations. They were the first to realize that the relative value of disinfectants depends very largely upon the conditions under which they work, and laid down the general laws that in any comparison of disinfectants close regard must be paid to the following conditions:

¹ The effect of treatment with a sulphide is not entirely due to the neutralisation of the HgCl_2 carried over in solution (*vide* p. 668).

- (1) Constancy of number and species of bacterium used.
- (2) Constancy of temperature.
- (3) Constancy of nutrient medium for test cultures.
- (4) Absence of other organic matter during disinfection.

They scrupulously observed all of these conditions, thus making their very exhaustive set of experiments the most valuable work which has yet appeared upon the subject of disinfection. A full description of the method as applied to the practical determination of the germicidal value of disinfectants was afterwards published by *Paul* (1901), anthrax spores and mercuric chloride being recommended as standard organism and disinfectant respectively.

Rideal and Walker (1903) published a method for the standardisation of disinfectants. They also realized the importance of the conditions of experiment. In their method (known as the "drop" method) a definite small amount of a broth culture of constant species and age was added to a constant volume of disinfectant solution. *B. typhosus* was chosen as the standard organism, and pure phenol as the standard disinfectant. By a series of trials with different concentrations both of disinfectant and pure phenol, under otherwise similar conditions, these authors determined the relative concentrations necessary to complete germicidal action in the same time. The ratio of the reciprocals of these concentrations was called the "carbolic acid coefficient" and was taken to express the germicidal value of the disinfectant in terms of carbolic acid as a standard.

This method has come into very general use, and been widely commended, among others by *Firth and Macfadyen* (1906) who found it to be superior in accuracy and convenience both to a method based on the "garnet" method of *Krönig and Paul* (1897) and the "thread" method of *Robert Koch* (1881).

The method of *Rideal and Walker*, in common with all preceding methods of standardisation, has been considered, in the opinion of many, to suffer from the disadvantage that whereas, in the majority of cases of practical disinfection, organic matter of some sort is present, there is no attempt to realize this condition during standardisation. Accordingly *Kenwood and Hewlett* (1906) and *Wynter Blyth* (1906) have suggested modifications of the *Rideal and Walker* method in which organic matter of different kinds is introduced; these modifications will be described later, in the section dealing with the effect of the presence of organic matter upon the efficiency of disinfectants.

CHAPTER II. CONDITIONS WHICH MUST BE COMPLIED WITH IN COMPARING THE GERMICIDAL VALUE OF DISINFECTANTS, AND THE MEANS SUGGESTED TO SATISFY THEM.

Disinfection has been shown by *Madsen and Nyman* (1907) and *H. Chick* (1908) to be a process exhibiting many analogies with a chemical reaction, one reagent being represented by the

bacterium and the second by the disinfectant. When the disinfectant is present in considerable excess the process proceeds in accordance with a definite law, the number of living bacteria per unit volume progressively and regularly decreasing with increase of time in a logarithmic ratio. This law is expressed by the equation

$$-\frac{dn}{dt} = Kn,$$

or
$$\frac{1}{t_2 - t_1} \log \frac{n_1}{n_2} = K,$$

where n_1 and n_2 are the numbers of bacteria surviving in unit volume after times t_1 and t_2 respectively.

In determining the relative germicidal value of disinfectants, whatever the procedure adopted, the temperature at which the disinfection takes place and the number in unit volume and resistance of the bacteria employed for the test must, as was pointed out by Krönig and Paul (1897, p. 3), be constant. If an end-point method is used, such as that of Rideal and Walker, the nutrient medium into which the test samples are withdrawn must not be altered during the progress of the experiment, and it is also necessary to maintain uniformity in the time allowed for the disinfectant to act.

The reasons which make constancy in these several points a necessity are discussed below.

1. *Temperature.* The velocity of disinfection has been shown to be influenced by temperature in accordance with the equation of Arrhenius. In the case of mercuric chloride and anthrax spores disinfection velocity was found to be increased 3 times for every 10° C. rise in temperature (Madsen and Nyman, 1907); in the case of disinfection of *B. paratyphosus* with metallic salts it was influenced to the same extent, but with phenol and emulsified disinfectants the velocity was increased 7—8 fold for every 10° C. rise in temperature (H. C. 1908, pp. 146 and 148). In consequence it is necessary that during any determination of germicidal value standard and disinfectant should be maintained at the same temperature, and germicidal values cannot be directly compared unless the temperature at which the determination was made is the same.

The general effect of rise in temperature in assisting disinfection has been known for some time (Koch (1881), Henle (1889), Behring (1890), Heider (1892)), and the necessity of keeping temperature constant during standardisation was insisted upon by Krönig and Paul (1897)

and by Paul (1902). In our own work 20° C. was fixed as a convenient temperature, and one approximating to the conditions of practical disinfection, and all experiments were made in a bath kept within half a degree of this temperature.

2. *Number of bacteria.* It follows from the law of disinfection formulated above that even when the disinfectant is present in great excess the greater the number of bacteria present in a unit volume the longer will be the time required for their disinfection. This effect may be seen from the instances in Table I below.

TABLE I.

Showing the effect upon the time taken for disinfection by phenol of varying the number of bacteria per unit volume.

B. paratyphosus 21° C.

Concentration of phenol parts per 1000	No. of organisms in unit volume	Time taken for disinfection, mins.
10	440,000	0·75
10	66,000,000	6·0
8	187,000	2·25
8	440,000	4·5
8	56,000,000	32·75
8	66,000,000	34·5
6	110,000	17·5
6	16,000,000	141

To obtain a constant result with a particular disinfectant, it is essential to work always with the same concentration of organisms, but in comparative experiments this is not necessary as long as they are consistent. Therefore, if the comparison of germicidal value is always made against a standard, the standard being tested at the same time against the same amount of culture, no inaccuracy results, even if the number of organisms should vary from time to time. Such variations are, however, extremely inconvenient, as they make it difficult to arrange beforehand experiments to yield the necessary negative and positive results.

A broth culture containing about 250 to 500 millions of *B. typhosus* per c.c. was obtained by sowing 6 c.c. of broth with a loopful from a fresh agar culture, and incubating for 24 hours at 37° C. Such a seeding

is an excessive one, so that although the number of bacilli sown may vary considerably, there are always enough added to obtain within 24 hours the maximum growth the medium can sustain.

3. *Resistance of the culture.* Any variation in the resistance of the culture although not introducing any error in experiments where disinfectant and standard are simultaneously tested is also inconvenient for the reason discussed in the preceding paragraph. It is therefore very important to obtain cultures of a standard resistance.

The difference in resistance between a culture freshly isolated and one grown for some time upon culture media will be referred to later (p. 677), but even in the case of a stock laboratory culture of *B. typhosus*, it was found that its resistance to phenol was considerably modified by its previous history. If such a stock culture were successively subcultured and maintained at 37° C., its resistance to disinfection was found to progressively increase; when kept in the cold room, after growth had taken place at room temperature, its resistance was found to be gradually lessened although in both cases the culture actually used for the experiment had been grown at 37° C.¹ It was found most satisfactory to grow and maintain the stock culture on sloped agar at room temperature (16—20° C.), subculturing about every two weeks. From such growths a standard loopful was sown in broth and cultivated for 24 hours at 37° C. By these means cultures of moderately constant resistance were obtained.

4. *Culture media.* It was found convenient, though not indispensable for accuracy in a comparative experiment (see above), to maintain the culture medium as constant as possible.

The exact nature of the medium employed for the test cultures, though not affecting the coefficient if both disinfectant and standard were simultaneously tested, was found to influence very much the actual result obtained. If as is the case with end-point methods, few, and possibly somewhat damaged, organisms be transferred to the culture tubes, the chances of growth are considerably greater in a more favourable medium. Thus the germicidal value of any disinfectant will apparently vary with variation in the medium employed for the test cultures, although the value relative to that of the standard phenol will remain constant. This is well shown in Table II.

¹ Rideal and Walker (1903, p. 431) concluded that increased temperature of incubation raised the resistance of *B. typhosus* to disinfectants. The data given are, however, insufficient, as no mention is made of the number of organisms, per unit volume being the same in the two experiments cited.

To obviate the variation due to unavoidable differences in beef broth, Brand's "Meat Juice" was substituted for fresh beef broth, and the following medium was adopted:

Brand's Meat Juice	10 c.c.	} in 1 litre tap-water.
Salt	5 gr.	
Peptone	10 gr.	
Glucose	10 gr.	

The medium had a reaction of +6 to +7 to phenolphthalein according to the notation of Eyre.

A large stock of the "Meat Juice" was put up in quantities of 10 c.c. and sterilised. The medium made with this preparation is not quite as favourable to the growth of *B. typhosus* as that made with fresh beef extract, but has been found to work well in practice.

TABLE II.

Influence of the suitability of the culture medium into which test samples are seeded upon the apparent germicidal value. The experiments were made with B. typhosus and all other conditions were identical.

	Disinfectant	Medium	Concentration required to kill in 15 minutes		Carbolic acid coefficient of the disinfectant
			Disinfectant parts per 1000	Pure phenol parts per 1000	
Exp. 23. 1. 08	I	Broth A	4.4	10.5	2.4
„ 31. 1. 08	„	Broth B	5.2	12.7	2.4
„ 23. 1. 08	II	Broth A	3.5	10.5	3.0
	„	Broth B	4.4	12.7	2.9
„ 17. 1. 08	III	Broth A	8	10.5	1.3
	„	Broth B	10	13	1.3
„ 23. 1. 08	IV	Broth A	3.5	10.5	3.0
„ 7. 2. 08	„	Broth B	4.8	13	2.7

5. *Time.* It has been shown by one of us (H. C. 1908) that a logarithmic relation exists between concentration of disinfectant and velocity of disinfection. This was found to be true in the case of such different organisms as *B. paratyphosus*, *Staphylococcus pyogenes aureus* and spores of *B. anthracis*, and such disinfectants as phenol, an emulsified disinfectant containing higher coal tar derivatives, and mercuric chloride (when the concentration of the metallic ions is taken into account). It follows that variations in concentration of disinfectant correspond to greater differences in the time taken for disinfection.

It was also found that the effect upon the time taken to kill of varying concentration is not the same with different classes of disinfectants. Two concentrations of two disinfectants, which are equally efficient, if allowed to operate for 5 minutes, do not necessarily bear the same relationship to one another as do two other concentrations of the same two disinfectants, which are equally efficient, if the time chosen for the test is 20 minutes. It follows that in comparing germicidal value in two cases where the effect of varying concentration upon the velocity of disinfection is very different (e.g. metallic salts and phenoloid bodies) completely different phenol coefficients will be obtained according to the time taken for the test, see Table III. If however, in comparing the germicidal value of two disinfectants, they are such, that the effect

TABLE III.

Showing the carbolic acid coefficients of mercuric chloride and silver nitrate, with variation in the time during which the disinfectant is allowed to act.

B. paratyphosus 20° C.

Disinfectant	Time of disinfection minutes	Concentration of disinfectant grm. per 1000	Carbolic acid coefficient
Mercuric chloride	2.5	0.88	13.6
Phenol	2.5	12	
Mercuric chloride	10	0.06	173
Phenol	10	10.4	
Mercuric chloride	30	0.018	550
Phenol	30	9.9	
Silver nitrate	2.5	0.068	176
Phenol	2.5	12	
Silver nitrate	10	0.015	693
Phenol	10	10.4	
Silver nitrate	50	0.009	922
Phenol	50	8.3	

of altering concentration upon the time taken for disinfection is about the same, it does not signify very much what time is taken for the test. An example of this is seen in the comparison of germicidal values of phenol and emulsified disinfectant "A" towards *B. paratyphosus* (see Table IV). On reference to a previous paper by one of us (H. C. 1908, pp. 119 and 122) it will be seen that the curves obtained by plotting concentration against time of disinfection are very similar for phenol and the coal tar disinfectant "A," and in the table

below (Table IV) the coefficients obtained by comparing the respective concentrations are the same within the error of such experiments.

TABLE IV.

Showing the constancy in the value of the carbolic acid coefficient of disinfectant 'A' with variation in the time during which the disinfectant is allowed to act.

<i>B. paratyphosus</i> 20° C.			
Disinfectant	Time of disinfection minutes	Concentration of disinfectant per 1000	Carbolic acid coefficient of disinfectant 'A'
'A'	2·5	0·8	15
Phenol		12	
'A'	12·5	0·6	17
Phenol		10·2	
'A'	40	0·5	16
Phenol		8·5	

It is obligatory, however, in any method for the standardisation of disinfectants, which shall be applicable to germicides of every class, to choose an arbitrary time, during which the disinfectant shall be allowed to act. What time shall be employed must be decided by considerations of hygiene, and convenience in working the test. Provided these conditions be satisfied, the time chosen should be such that the test be not unduly prejudicial to any particular class of disinfectant. As previously shown, as far as the relationship between the germicidal efficiency of phenol and the emulsified disinfectants containing higher tar acids is concerned, it is immaterial whether the comparison be made after 10 minutes or 30 minutes, but the shorter time is greatly to the disadvantage of mercury and silver salts. We are of the opinion that for practical purposes it is desirable that a disinfection be completed in about half an hour, and this time is quite a convenient one for the laboratory tests. The employment of a shorter interval for the tests would be too unfavourable to metallic salts such as those of mercury and silver, which are undoubtedly amongst the most powerful germicides we possess.

Metallic salts are effective in very small concentration (1000 times less than phenol) and have the peculiarity that, if the concentration is increased, although the velocity of disinfection is increased also, this does not occur to nearly the same extent as with other disinfectants (H. C. 1908, Tables XVII, XIX and XXII). In the case of HgCl_2 this

is partly but not altogether¹ accounted for by the fact that the Hg^{++} ions are the real disinfecting agent and owing to peculiarities in the ionisation of mercuric salts, changes in concentration of the salts influence the concentration of the ions comparatively little.

Compared with phenol, the action of HgCl_2 upon bacteria is slow. For example, it was found that *B. paratyphosus* (6,000,000 per c.c.) could withstand the action of 5% HgCl_2 for more than four minutes (H. C. 1908, p. 126), and *Staphylococcus pyogenes aureus* in similar concentration for more than 15 minutes (see Table VI, below) if immediately treated with a sulphide solution. This appears to be due to delay in HgCl_2 actually "getting to work" upon the contents of the bacterium, and will be discussed later.

Activity in high dilution is a valuable characteristic in a disinfectant. From this point of view mercury and silver are preeminent amongst chemical disinfectants, and it is advisable to choose a time for the standardisation which shall to some extent measure this property. When we embarked upon this enquiry we did not realise to what extent the metallic salts would suffer by comparison when short times were allowed for disinfection, and for most of our work 15 minutes was the time during which the disinfectant was allowed to act. We think however that, for the consideration discussed above, the time should be extended to 30 minutes.

CHAPTER III. MODIFICATION OF RIDEAL-WALKER METHOD NECESSITATED BY ADOPTING A CONSTANT TIME.

The introduction of an arbitrary time, which is necessitated by the considerations dealt with above, involves a modification in the method recommended by Rideal and Walker; unfortunately this modification makes the determination more tedious to perform.

The procedure we adopt is as follows:

Everything used in the experiment, tubes, pipettes, etc., being previously sterilised, a series of tubes containing 5 c.c. of the disinfectant in different concentrations are placed in a water bath at 20° C. When the tubes have taken the temperature of the bath, they are one after another inoculated with five drops of 24 hours' culture of *B. typhosus* from a standard pipette², the time being registered by a chronograph.

¹ Watson (1908). Constant K for HgCl_2 , when Hg^{++} ions are reckoned as disinfectant, = 3.8, for phenol $K=5.5$.

² H. C. 1908, p. 96.

Exactly one minute is allowed to pass between each inoculation. When 30 minutes have elapsed since the first tube was inoculated, samples in duplicate are taken from it with a platinum loop¹, and sown in 10 c.c. glucose broth containing litmus (see p. 662). One minute later the second tube is sampled and so on. These test cultures are incubated at 37° C. and always kept four days under observation.

Supposing the value of the disinfectant to be tested is totally unknown, the first series of observations must be scattered over a wide range, e.g. concentrations from 1 in 10 to 1 in 10,000. Having ascertained that the concentration necessary to kill in 30 minutes is between, say, 10 in 1000 and 1 in 1000 the second series is arranged to narrow it down to between, say, 4 and 5 per 1000, and a third series may determine the necessary concentration as between 4·2 and 4·5 per 1000. At this last trial a series of tubes, containing various strengths of pure phenol, are simultaneously tested.

The Rideal-Walker method, when employed to test disinfectants containing the higher coal tar derivatives, has been criticised (Gruber, (1891), Proskauer (1907), Seligmann (1907)), in view of the carrying-over of traces of disinfectant with the test samples, which may cause inhibition of growth in the test cultures. When disinfectants containing metallic salts are in question this carrying-over is of great importance and the traces thus added to the test cultures have to be neutralised. This is conveniently done by the addition of sulphides (see below, p. 668 where the subject is treated in detail). In the case of phenol and coal tar disinfectants there is, however, no suitable neutralising agent, but special experiments made for the purpose showed that there was no error introduced in carrying-over small quantities of these disinfectants. For example, a concentration of 1 in 1000 phenol in the test culture did not inhibit growth either of 10,000 or of 40 organisms in 10 c.c. In the case of disinfectant "B" a concentration of 1 in 10,000 was necessary to cause inhibition of the same concentration of organisms; 1 in 100,000

¹ A platinum loop of standard size was used for sampling. It is important to make the sample as large as possible to avoid errors in sampling, consequently the loop was bent horizontally and removed parallel to the surface of the liquid; it was found that the sample was much larger than when the plane of the loop was removed at right angles to the surface of the liquid. According as the sample taken is large or small, the determination is concerned with more or less complete disinfection. The two loopfuls removed (= about 0·0086 gr.) constitute about $\frac{1}{300}$ of the total volume, 5 c.c., and, if the test sample shows no growth, this indicates that the original number, about 30 millions, is reduced to less than 300 (cf. H. C. 1908, p. 118, where a larger sample was withdrawn (0·08 c.c.) and no growth indicated that about 30,000,000 per 5 c.c. had been reduced to less than 60 per 5 c.c.). For practical purposes disinfection may be regarded as complete in either case.

caused no inhibition in either case (see Table V below). These concentrations are higher than any attained in the test cultures in the course of a standardisation experiment.

CHAPTER IV. SPECIAL CONSIDERATIONS INVOLVED IN THE CASE OF DISINFECTANTS CONTAINING MERCURY.

In the standardisation of disinfectants containing mercuric chloride and some other salts of heavy metals some complications are encountered which are not met with in the case of the organic disinfectants. Disinfection by salts of mercury exhibits the following two peculiarities.

1. *Mercuric chloride is capable of acting as a germicide even in high dilution (1 in 1,000,000) if sufficient time be allowed for the action.*

Robert Koch (1881) found that 3 in 1,000,000 mercuric chloride inhibited growth of anthrax spores; Geppert (according to Behring) found that a concentration of 1 in 2,000,000 was sufficient to inhibit growth of anthrax spores; Behring himself found that 1 in 400,000 mercuric chloride was needed to prevent growth of anthrax spores.

TABLE V.

Showing the comparative inhibiting power of disinfectants upon the growth of some organisms.

Date of exp.	Test organisms	Disinfectant	Concentration inhibiting growth, parts per million	Concentration not inhibiting growth, parts per million	No. of organisms sown in 10 c.c.
15. 1. 07	<i>B. paratyphosus</i>	Mercuric chloride	1	0.1	1020
30. 6. 08	„	„	—	1	5000
28. 5. 08	<i>Staphylococcus pyogenes aureus</i>	„	34	17	5000
			34		3,000,000 to 4,000,000
28. 5. 08	<i>B. pestis</i>	„	1.7	—	2000
15. 3. 07	<i>B. paratyphosus</i>	Silver nitrate	1.4	0.14	40 to 80
20. 7. 08	<i>B. typhosus</i>	Phenol	—	1000	40 or 10,000
20. 7. 08	„	Disinfectant B	100	10	40 or 10,000

We have made some determinations of these amounts with different test organisms and a few results with different disinfectants are arranged in Table V, for purposes of easy comparison. The results with mercuric chloride are roughly in accord with those of Koch and Geppert, and also

show the influence of the quantity of bacteria introduced upon the result. This difference may be due to selection; the more bacteria introduced, the greater will be the number of specially robust individuals present. Or, there may be an exhaustion of the mercuric chloride owing to the number introduced. Possibly the result may be due to a combination of both effects.

In consequence of this germicidal action in high dilution it is necessary, as originally insisted upon by Geppert, to precipitate the mercury carried over with samples withdrawn during an experiment, as this would otherwise prevent growth in the test cultures. A sulphide is the common precipitant employed and it has been found necessary to add an excess over and above the amount calculated for combination with the mercury carried over (H. C. 1908, p. 123, Tab. XX). The reason for this excess will be discussed when dealing with a second peculiarity of disinfection by mercuric salts. This treatment with sulphide means a little extra trouble, for such precipitants have themselves an inhibiting action upon the growth of bacteria, and it is important not to add enough to interfere with subsequent growth. A saturated solution of hydrogen sulphide¹ in distilled water was found to be the most satisfactory precipitant and 0.2 c.c. added to 10 c.c. of broth (see H. C. 1908, pp. 123—125) was found ample for neutralisation and not sufficient to interfere with subsequent growth.

2. *Organisms submitted to the action of mercuric salts and subsequently washed free of the disinfectant, may not grow if planted directly into broth, but are not necessarily damaged irretrievably, and if treated with a sulphide solution, a certain proportion can be resuscitated.*

These facts have already been recorded by one of us (H. C. 1908, p. 132), and it was suggested that they were due to the formation of a mercury addition-compound with the substance of the bacterium, which prevented the organism manifesting its vitality by growth, until it was decomposed by the action of a sulphide.

The following series of experiments demonstrates the correctness of this interpretation.

To an emulsion of *Staphylococcus aureus* sufficient HgCl_2 was added to make a concentration of 1%. The mixture was immediately decanted into a series of tubes and placed in a rapidly rotating centrifuge ($t = 18 - 20^\circ \text{C.}$). After 13 minutes the supernatant fluid was removed from the tubes and the deposited bacteria thoroughly

¹ The H_2S solution must be fresh.

washed with distilled water in the centrifuge. The water was withdrawn and the tube filled with nutrient broth and incubated. In no case was growth observed. In a second tube the bacteria, after removal of the mercury solution, were at once treated with 1/10th saturated H_2S water. After ten minutes the sulphide solution was removed and the tube filled with broth; on incubation growth occurred. In the case of five other tubes, varying intervals up to five hours elapsed before the sulphide was added, with the result that the addition of sulphide was found to be effective up to 77 minutes after the treatment with mercuric chloride. The addition of the sulphide blackened the washed bacterial deposit, showing that some mercuric salt had been absorbed by the bacterium and formed a compound with its substance. With the mercury attached, subsequent manifestation of life by multiplication is in abeyance unless a large excess of sulphide be applied as antidote.

From the above observations it follows that in determining the germicidal value of HgCl_2 (and the same holds for preparations containing salts of the heavy metals) we are confronted with the difficulty of deciding when the bacterium is to be considered for practical purposes dead¹. Bacteria which have been subjected to the action of dilute solutions of HgCl_2 will not multiply unless they come into contact with SH_2 or soluble sulphides. When used for surgical purposes, where there is little chance that the action of the HgCl_2 may be reversed by contact with sulphides, mercuric chloride will prove effective even in very great dilution. Should, however, the disinfected material subsequently come in contact with decomposing organic matter, animal dejecta, etc., mercuric chloride will prove less efficient.

It is impossible to institute an accurate comparison between the value of metallic salts and organic disinfectants of the type of phenol. If, in estimating the germicidal value of mercury or silver salts, we omit to treat the sample of liquid containing the bacteria with a sulphide at the expiration of the time allowed for disinfection, we shall form a greatly exaggerated conception of their efficiency, whereas their value will be somewhat underestimated if this procedure be adopted. The latter method is now commonly employed. This escapes the

¹ Most divergent results have been obtained with mercuric chloride. R. Koch (1881), who was the first to lay stress upon the powerful germicidal value of mercuric chloride, obtained an exaggerated idea of its worth because he employed no precipitant to neutralise the traces of sublimate carried over into his test cultures. Geppert (1889 and 1891) pointed this out, but it is probable that he also overestimated the germicidal properties of HgCl_2 because, although he neutralised the traces of sublimate carried over, he did not employ a sufficient excess of sulphide.

grosser error but affords a severe judgement upon the value of metallic salts when used under certain conditions.

Summary of Chapters II, III, and IV.

1. In any method of standardisation it is necessary that the test shall be carried out at a constant temperature, as the disinfection process has a high temperature coefficient. For any method to be of general application, it is also necessary that the temperature selected shall be adhered to in all determinations, since the temperature coefficient of disinfection varies for different disinfectants. The temperature adopted was 20° C.

2. To avoid unnecessary labour, the number per unit volume and resistance of the bacteria employed for the test should be kept as constant as possible, and the culture medium used both for the original culture and for the test subcultures should be maintained of constant composition.

Neglect of these precautions does not affect the value found for the carbolic acid coefficient, if disinfectant and phenol are simultaneously tested, but leads to much trouble and inconvenience in the practice of standardisation.

3. It is necessary to arbitrarily select a fixed time during which the disinfectants shall be allowed to act.

A logarithmic relation exists between concentration of disinfectant and velocity of disinfection, and the effect of varying concentration upon the time taken to kill, is different for different disinfectants. Different phenol coefficients may be obtained according to the time adopted in the test.

The time suggested is 30 minutes.

4. In the case of metallic salts, a sulphide must be employed to neutralise the traces of disinfectant carried over with the test sample. With other disinfectants neutralisation was found to be unnecessary.

5. In the case of mercuric chloride, it was found necessary to add a large excess of sulphide over and above that required for combination with the mercury carried over, in order to decompose a compound formed between the mercuric salt and the substance of the bacterium. The presence of this compound prevents manifestation of vitality on the part of the organism unless an excess of soluble sulphide be administered as an antidote.

CHAPTER V. CHOICE OF TEST ORGANISM.

An important advance, made by Koch (1881) upon the method of his predecessors, Pringle (1750), Baxter (1875), L. Buchholtz (1875), and Jalan de la Croix (1881), was the use of pure cultures of bacteria for the determination of germicidal values of disinfectants instead of mixtures of putrefactive bacteria. He employed the spores of *B. anthracis*. Spores are much more resistant to disinfection than most vegetative forms, and many disinfectants which may usefully be employed for the latter are practically valueless against the former, so that Koch's test demanded a high standard of efficiency.

The question arises whether, for practical purposes, such a high standard is necessary. Rideal and Walker's method, which is largely used in this country, and which adopts carbolic acid and *B. typhosus* as standard disinfectant and test organism respectively, assumes that this question may be answered in the negative.

At the time when Koch made his experiments the number of pathogenic organisms, which formed spores, was overestimated. At the present time it is known that the germs of the great majority of diseases to which man is liable are produced by vegetative forms. It must however be remembered that there are a number of diseases about the etiology of which we have no data (e.g. typhus, scarlet fever, measles, etc.) and also several others, common to man and animals, which are known to be caused by spore-bearing organisms (e.g. tetanus, anthrax, malignant oedema, etc.). A disinfectant may at any time be required to obviate risk of infection from any of such diseases, and the departure from the high standard of Koch (mercuric chloride and anthrax as standard disinfectant and test organism respectively) and the adoption of a low standard, such as that of Rideal and Walker (carbolic acid and *B. typhosus*) may give rise to a false sense of security unless it is clearly understood what has been done.

*Variation in the resistance of different vegetative forms to
three different types of disinfectant.*

The types of organisms chosen were (1) *B. typhosus* and *B. paratyphosus*, (2) *Staphylococcus pyogenes aureus*, (3) *B. pestis*. In each case 5 drops (·086 c.c.) from a standard pipette¹ taken from a 24 hours'

¹ See H. Chick (1908, p. 96).

culture at 37° C. (30° in the case of *B. pestis*) were added to 5 c.c. disinfectant solution.

The disinfectants used were phenol, emulsified disinfectant B, mercuric chloride, and oxychinoline sulphonate of potassium ("chinosol").

In each case determination was made of the concentration of disinfectant required to kill the amount of culture added in 15 minutes. The details of the method, which is the one ultimately adopted for the standardisation of disinfectants, has been described in detail (p. 666); it will be sufficient here to give the results obtained (Table VI).

TABLE VI.

		Concentration, parts per 1000, required for disinfection in 15 minutes						
		Test organ- isms	Pure phenol	Disinfect- ant B	Relative conc. of Hg++ ions	Mercuric chloride ¹	Oxychino- line sulpho- nate of potassium	Carbolic acid coeffi- cient
Exp. 20.	3. 08	<i>B. typhosus</i>	7.9	0.51	—	—	—	15.5
,,	11. 3. 08	,,	8.2	0.54	—	—	—	15.2
,,	22. 5. 08	<i>Staphylococcus pyogenes aureus</i>	9.0	2.0	—	—	—	4.5
,,	28. 5. 08	,, ,	10.5	2.4	—	—	—	4.4
,,	19. 5. 08	<i>B. pestis</i>	8.5	0.2	—	—	—	40
,,	3. 6. 08	,,	7.25	0.18	—	—	—	40.3
,,	14.11.07	<i>B. paratyphosus</i>	8.00	—	about 42.5	about 0.1	—	about 80
,,	21. 5. 08	<i>B. typhosus</i>	7.75	—	46.0	0.15	—	81
,,	2. 6. 08	<i>Staphylococcus pyogenes aureus</i>	10.5	—	more than 107.5	more than 50	less than	0.21
,,	2. 6. 08	,, ,	10.5	—	do.	do.	— ,,	0.21
,,	16. 6. 08	,, ,	10.5	—	do.	do.	— ,,	0.21
,,	16. 6. 08	,, ,	9.5	—	do.	do.	— ,,	0.19
,,	3. 6. 08	<i>B. pestis</i>	7.25	—	33.5	0.035	—	207
,,	22. 8. 07	<i>B. typhosus</i>	6.5	—	—	—	26	0.25
,,	23. 9. 07	<i>Staphylococcus pyogenes aureus</i>	9.0	—	—	—	10	0.9

It is very noticeable that, whereas the strengths of carbolic acid required to kill the three types of organism chosen varies comparatively

¹ H₂S was used for neutralising traces of HgCl₂ carried over with the sample into the test culture at the end of the 15 minutes.

little (see column 2, Table VI), *B. pestis* to a large degree and *B. typhosus* to a less degree show themselves more susceptible to the action both of emulsified disinfectant "B" and mercuric chloride. In the case of "chinosol," the reverse is true, *Staphylococcus aureus* being found more susceptible than *B. typhosus* to the disinfectant.

The determinations of concentration of HgCl_2 necessary for disinfection in 15 minutes (Table VI column 5), and therefore its carbolic acid coefficients are merely approximate. This is owing to the fact that it is not the concentration of HgCl_2 as such which is operative, but the concentration of mercuric ions (Krönig and Paul (1897), H. Chick (1908)). The ionisation of mercuric salts is peculiar and 100% increase in concentration of the salt, e.g. from 1 to 2 parts per 10,000, only increases the concentration of ions by 15%, an amount which does not greatly exceed the experimental error of the determination. The numbers representing the concentration of mercuric ions corresponding to various concentrations of mercuric salt, given in Table VI, were obtained from a curve constructed by means of the results of Luther (1904) and Kahlenberg (1901).

Whereas very low concentrations of mercuric chloride are effective against *B. typhosus*, *B. paratyphosus* or *B. pestis* (see Table VI), with the method employed, viz.—treatment with H_2S at the end of 15 minutes—it was found impossible to kill *Staphylococcus pyogenes aureus* by treatment with 5% HgCl_2 for this length of time. 5% of mercuric chloride is a nearly saturated solution, so that a determination of the carbolic acid coefficient of mercuric chloride could not be made.

Comparative resistance to disinfectants of spores and vegetative forms.

It is notorious that spores are much less readily destroyed by disinfectants than vegetative forms, and we have tried to ascertain the relation between the strengths of some disinfectants necessary for the destruction of one and the other.

In order to compare by direct experiment the relative strengths of any given disinfectant required to kill (a) spores and (b) vegetative forms it would be necessary that approximately the *same number per unit volume* of individuals of either class should be killed in the same time. The relative strengths of disinfectant in the two cases would then be an indication of the relative resistances of the particular spore and vegetative form. It would however be extremely difficult to make

such an experiment, the necessary conditions could only be obtained by accident and this never has occurred in the course of an extensive series of experiments. The result could however be calculated from the relation which has been shown to exist between the concentration of disinfectant and time taken for disinfection. Unfortunately we have no available data of our own except in the case of phenol.

Experiments with phenol. B. paratyphosus and anthrax spores. Experiments have been published by one of us in which anthrax spores were destroyed by phenol, and the progress of the reaction was studied by enumerating the spores surviving in unit volume at successive intervals of time. In one such experiment with 5% phenol at 20° C. (H. C. 1908, p. 97) it was found that 434 anthrax spores in unit volume (a standard drop) of the solution were reduced to 28 in 25·5 hours.

Exactly similar experiments were made with phenol and *B. paratyphosus*, at the same temperature. In one such experiment with 0·6% phenol it was found that 484 bacteria in unit volume were reduced to 28·5 in 4 minutes (H. C. 1908, p. 106); in a second similar experiment 484 were reduced to 24 in 4·5 minutes (p. 111). It is therefore not very inaccurate to say that in the case of *B. paratyphosus* and 0·6% phenol, 434 bacteria per unit volume would be reduced to 28 in about 4·25 minutes.

In these three experiments the concentration of bacteria was almost identical. If however this had not been so, the actual time necessary for a reduction in numbers of *B. paratyphosus* similar to that of the anthrax spores could have been obtained from the drawn curves showing rate of decrease of numbers of *B. paratyphosus* during disinfection with 0·6% phenol (see figs. 7 and 9, H. C. 1908, p. 105) or from the formula expressing the same relation (p. 95).

The two series of experiments are still not directly comparable, for the time of disinfection of the anthrax spores was 25·5 hours and that of the similar reduction in number of *B. paratyphosus* was 4·25 minutes. It is necessary to find out what concentration of phenol would perform the disinfection of the same number of *B. paratyphosus* in 25·5 hours.

The logarithmic relation existing between the concentration of a disinfectant and the time taken for disinfection has recently been suitably expressed by H. E. Watson (1908) by the equation.

$$K \log C + \log t = \text{constant},$$

where K is some constant depending on the nature of the disinfectant

and in some cases also of the organism employed. For carbolic acid and *B. paratyphosus*,

$$K = 5.5,$$

and we have the equation

$$5.5 \log C + \log t = \text{constant}.$$

Therefore $5.5 \log 0.6 + \log 4.25 = 5.5 \log x + \log 1530$

from which $x = 0.206$,

and the concentration is 0.206%. The ratio of the concentration required to kill anthrax spores to that required in the case of *B. paratyphosus* is as 5 to 0.2, that is 25 times as strong.

Staphylococcus pyogenes aureus and *anthrax* spores. Krönig and Paul (1897) and Paul and Prall (1907) have made enumeration experiments with phenol upon anthrax spores and *Staphylococcus pyogenes aureus* respectively, and among these we have found two sets of observations where the reduction in numbers of the two species happens to be almost identical.

It is, strictly speaking, not allowable to institute a comparison between two such experiments, as no data are to be obtained as to the concentration of the bacteria during disinfection. Both sets of authors employed the garnet method (Krönig and Paul, 1897, pp. 7—11), in which the organisms are submitted to the action of the disinfectant in a thin film dried upon the surface of the garnets. For any series of experiments, in which the same emulsion of organisms is used for preparing the garnets in the first instance, it may be concluded that the concentration of bacteria during disinfection will be constant¹ also. It is less permissible to compare experiments made with different organisms, and at different times, even though the details of the method remain the same. At the same time, the probability is that, since in both cases the concentration of bacteria approached a maximum, we shall not obtain very erroneous results by comparing the two experiments.

In the one case (Krönig and Paul, 1897, p. 93) about 1000 anthrax spores were reduced to 260 in 25 hours by 5% phenol at 18° C.; in the other (Paul and Prall, 1907, p. 116) 1040 staphylococci were reduced to 205 in 2½ minutes by 0.94% phenol at 18° C.

¹ That this is the case is shown by the fact that the laws shown to govern the disinfection process have been as accurately deduced from experiments using the garnet method (Krönig and Paul (1897), Madsen and Nyman (1907)) as from those in which the constancy of the concentration of bacteria was assured (H. C. 1908).

By calculation from the equation $5.5^1 \log C + \log t = \text{constant}$, it appears that 0.29% carbolic would reduce 1040 staphylococci to 205 in 25 hours, so that the relative concentration is approximately as 17 to 1. This figure is in fair agreement with the calculated result in the case of paratyphoid, for, as previously shown (Table VI), staphylococci possess a somewhat greater resistance towards phenol.

Mercuric chloride. It has not been possible to obtain any perfectly comparable data as to the comparative resistance of spores and vegetative forms towards mercuric chloride. However, with the reservation mentioned above, we propose to use again two sets of experiments with the garnet method in which anthrax spores and *Staphylococcus pyogenes aureus* respectively were disinfected.

TABLE VII.

Exp.		Test organism	Concentration of
			disinfectant B necessary to kill in 15 minutes, parts per 1000
Exp. 28.	5. 08	<i>Staphylococcus pyogenes aureus</i> A, old laboratory culture	2.0
„	22. 5. 08	<i>Staphylococcus pyogenes aureus</i> B, viru- lent culture	2.4
„	4. 6. 08	<i>B. typhosus</i> A, old laboratory culture ...	0.55
„	4. 6. 08	<i>B. typhosus</i> B, which had then recently been passaged	0.80
„	15. 7. 08	<i>B. typhosus</i> A	0.59
„	15. 7. 08	<i>B. typhosus</i> B after 41 days' culture upon artificial media	0.56

In the former case 82 anthrax spores were reduced to 19 in 2 minutes by 1.69% mercuric chloride at 18° C. (Krönig and Paul, 1897, p. 24). In the latter case, from three experiments (Paul and Prall, 1907, pp. 103, 104, and 105) 98 staphylococci were reduced to about 22 by 0.053% mercuric chloride, also in 2 minutes at 18° C.

In this instance temperature of disinfection, the number (and presumably the concentration) of bacteria disinfected and the time taken all being the same, comparison of the concentration of disinfectant in the two cases gives direct indication of the relative resistance to mercuric chloride of anthrax spores and *Staphylococcus pyogenes aureus*; this ratio is as 32 to 1².

¹ The constant K happens to be the same in the case of phenol for both *B. paratyphosus* and *Staphylococcus pyogenes aureus*.

² In the case of mercuric chloride, it has been shown that mercuric ions are the real disinfecting agent (Krönig and Paul, 1897; H. Chick, 1908; Watson, 1908). Figures

Differences in resistance to disinfectants between virulent and avirulent strains of the same organism.

After cultivation upon artificial media a certain loss of resistance towards disinfectants was noticed in the case of *B. typhosus* and *Staphylococcus pyogenes aureus*, when compared with freshly isolated virulent strains. The experimental results are set forth in Table VII above.

Summary of Chapter V.

1. In the case of vegetative organisms a disinfectant varies in efficiency as much as ten times according to the organism against which it is tested. Some disinfectants are more efficient against one vegetative species of bacteria, others against another.

2. In the case of spores, metallic salts are the most efficient germicides. The action of phenol and emulsified disinfectants is too feeble for practical use.

3. The ratio between the concentration of phenol required to kill the same number per unit volume of sporing and vegetative forms in the same time varied according to the particular organism employed between 17 and 25 to 1.

4. A virulent strain of any particular species is generally somewhat more difficult to kill than a non-virulent strain.

5. Owing to the want of constancy as regards disinfection shown by various species, it is necessary to fix upon one particular organism for use in testing the germicidal value of disinfectants.

6. *B. typhosus* occupies an intermediate position in many respects, and has come to be employed in this country for the standardisation of disinfectants. It possesses the advantage of forming a fairly uniform suspension in broth culture and it is an organism which it is frequently desired to destroy in practice.

7. There is no particular reason why a virulent strain of *B. typhosus* should be employed in standardisation, and its use for the purpose would entail an unnecessary amount of danger.

CHAPTER VI. INFLUENCE OF ORGANIC MATTER.

(1) *Introductory.*

The practical value of the results obtained by any method of standardising disinfectants may justly be questioned if the comparison is expressing the relative concentration of Hg^{++} ions, corresponding to concentrations of the salt in the above experiments, were obtained from the results of Kahlenberg (1901) and Luther (1904); the ratio of the concentrations of Hg^{++} ions in the two cases is as 1.5 to 1.

made in the absence of organic matter, since in practice disinfectants are commonly used in its presence. The importance of this is evident when it is realised that, although presence of organic matter lessens the efficiency of every chemical disinfectant with which we have worked, the interference is to a different degree in every special case, depending (1) on the chemical nature and physical condition of the disinfectant, and (2) on the nature and condition of the organic matter.

Kenwood and Hewlett (1906) showed that in the presence of urine and faeces the value of phenol (Rideal-Walker method) was barely impaired, while emulsified disinfectants were reduced to two-thirds and one-half of their original value. These authors therefore suggest that the Rideal-Walker coefficient may in certain cases give a misleading value and that in any useful method of standardisation organic matter should be introduced. The sum of opinion, as the result of a long controversy¹ lasting over the last few years, is that it is desirable to introduce organic matter into any standard method of comparing the germicidal value of disinfectants, but great difficulty has been experienced in selecting the most suitable material.

It is impossible to arbitrarily select some convenient organic material without doing injustice to one or other class of disinfectant, so that one has to fall back upon some form which the disinfectant may encounter in practice. The use of faeces has the advantage of an approximation to the conditions of disinfection in many cases; it has, however, been objected to by Somerville and Walker (1906, I and II) as being too irregular in composition and therefore too uncertain in its effect upon disinfection to be adopted. Somerville and Walker suggest in a later paper (1907) the introduction of some standard organic matter, such as protein or gelatin, *in solution*.

In the case of many disinfectants, however, the physical condition of the organic matter present is an important factor in its effect upon the disinfectant, so that any method of standardisation which introduced organic matter only in solution would be inadequate, as in practice contaminating organic matter is partly in a particulate form. Wynter Blyth (1906) considers the question of organic matter both in solution and suspension, and notes the especial effect of the latter (e.g. faeces) in reducing the efficiency of emulsified disinfectants. He considers faeces, however, to be too inconstant in composition to be included in any standard test, but finds that a similar effect is produced

¹ Full abstract is to be found in *Public Health Engineer*, May—Aug. 1906, and in the *Local Government Officer*, Sept. 1906 to May 1907.

by the addition of milk and recommends that milk should be adopted as a standard organic matter on the score of its convenience and approximate constancy in composition.

We have made experiments to determine the effect of the presence of various kinds of organic matter both in solution and in suspension upon the germicidal value of a variety of disinfectants.

These experiments were undertaken with the view to ascertain how far the efficiency of disinfectants was modified by the conditions obtaining when they are added as a second line of defence to sera, vaccines, etc.; or when employed to disinfect dejecta.

Two methods were employed for estimating the effect of organic matter upon the germicidal value of disinfectants.

Method 1. In these experiments the time of disinfection was constant. Two concentrations (C_1 and C_2) which disinfected in 15 minutes, in distilled water and in presence of organic matter respectively, were directly determined, and the relative efficiency of the disinfectant in the two cases expressed as the ratio $\frac{C_2}{C_1}$.

Method 2. In the case of mercuric chloride we had not sufficient data available for an adequate investigation of the effect of serum in different concentrations and a second method was adopted which was less direct and involved rather more calculation.

The concentration (C_1) of a disinfectant was kept constant and the time (t_1) of disinfection of a certain number of organisms in unit volume was measured, when no organic matter was present, and the time (t_2) was also measured, when organic matter was present. It is possible to estimate the concentration C_2 of disinfectant, which would kill in the time t_2 , if no organic matter had been added, by means of the curves showing the relation between concentration and time taken (H. C., 1908), or the result can be calculated by means of the formula $K \log C + \log t = K$, which has been shown by Watson to express these curves. The ratio $\frac{C_2}{C_1}$ expresses the efficiency of the disinfectant in presence of the organic matter if the efficiency in distilled water is reckoned as unity.

The following is an example of the calculation of relative efficiency in a case where the addition of organic matter was in the form of 10 % blood serum. In an experiment with phenol and *B. paratyphosus* it was found that 10 per 1000 phenol disinfected 30 millions of *B. paratyphosus* in 5 c.c. in 3 minutes. When 10 % serum was present the time taken was 12.5 minutes. Watson (1908) has shown that in the experiments of one of us with phenol and *B. paratyphosus* $5.5 \log C + \log t = \text{constant}$.

Therefore $5.5 \log 10 + \log 3 = 5.5 \log C_2 + \log 12.5$, where C_2 = concentration of phenol which would disinfect in 12.5 minutes if no serum had been present. Solving this equation we obtain $C_2 = 7.7$, and the relative efficiency of phenol with and without 10 % blood serum, $\frac{C_2}{C_1} = 0.77$.

(2) Organic matter in solution.

Blood serum was chosen as a type of soluble organic matter which would be present in certain cases of disinfection. Its influence upon the germicidal value of disinfectants when present in concentrated form is also important, because disinfectants are frequently added to antitoxic sera as an additional precaution against contamination.

Experiments with 10 % blood serum. The effect of 10 % blood serum upon the efficiency of disinfectants was investigated by the two methods already described.

The effect of serum was determined in the case of phenol, mercuric chloride and the emulsified disinfectant "B," using *B. paratyphosus* and *B. typhosus* as test organisms. The results obtained are set forth in the tables below.

TABLE VIII.

Showing relative efficiency of various disinfectants with and without the addition of 10 % blood serum.

Experiments made with Method 1.

Date of exp.	Test organisms	Disinfectant	Concentration required to kill in 15 minutes, parts per 1000		Relative efficiency, (concentration required in distilled water = 1)
			In distilled water	In presence of 10 % serum	
12. 11. 07	<i>B. paratyphosus</i>	Phenol	8	9	0.89
14. 11. 07	"	"	7.75	8.25	0.94
26. 5. 08	"	"	8.75	10.5	0.83
21. 5. 08	<i>B. typhosus</i>	"	7.75	9.5	0.87
13. 11. 07	<i>B. paratyphosus</i>	Disinfectant "B"	0.7	1.0	0.70
15. 11. 07	"	"	0.625	0.85	0.77
15. 11. 07	"	Mercuric chloride *	about 0.05	about 0.45	about 0.11
	"	"	" 0.10	" 0.40	" 0.25

* For reasons explained above, p. 673, the error in the determination of the germicidal value of mercuric chloride is 100 %.

TABLE IX.

Showing the relative efficiency of mercuric chloride when employed as disinfectant in the presence of various concentrations of blood serum.

Experiments made with Method 2.

Organism	Initial concentration of HgCl_2 parts per 1000	Nos. expressing initial concentration of Hg^{++} ions	Concentration of blood serum %	Time taken for disinfection, minutes	Nos. expressing effective concentration of Hg^{++} ions	Relative efficiency in terms of concentration of Hg^{++} ions	Effective concentration of HgCl_2 parts per 1000	Relative efficiency in terms of concentration of HgCl_2
Exp. 9. 11. 07.								
<i>B. paratyphosus</i>	0.5	57.5	—	7.2	57.5	1.0	0.5	—
	0.5	57.5	5	10	52.7	0.91	0.30	0.60
	0.5	57.5	10	14.2	48.1	0.84	0.18	0.36
	0.5	57.5	20	39	36.9	0.64	0.05	0.10
	0.5	57.5	30	62	32.6	0.56	0.03	0.06

In the case of phenol the efficiency of the disinfectant is reduced about 12 % in the presence of 10 % blood serum, and with the emulsified disinfectant "B," the reduction is rather greater. The results with corrosive sublimate (see Table IX) require a few words of explanation. In calculating the concentration of HgCl_2 , which in the absence of serum would disinfect in the time observed, it is necessary to express the original concentration of sublimate in terms of concentration of Hg^{++} ions. This was done by means of a curve constructed from the figures of Luther and Kahlenberg already referred to (p. 677 footnote). The calculated concentration, which in the absence of the serum would operate in the same time, was therefore also in terms of concentration of Hg^{++} ions and had to be again translated back into concentration of HgCl_2 by the same means. The effect of adding blood serum to a solution HgCl_2 is to precipitate some of the mercuric salt as an albuminate which thereby lowers the concentration of mercuric chloride, but, owing to the peculiar ionisation of this salt, the concentration of Hg^{++} ions varies but slowly with alteration in concentration of the salt.

Effect of blood serum in high concentration. A few experiments were made with *B. paratyphosus*, but this organism was abandoned because it was found to be particularly susceptible to the bactericidal action of horse serum. This was still very marked after the serum had been heated to 60° C. for one hour.

The effect of serum in high concentration upon the germicidal value of phenol was therefore studied with *Staphylococcus pyogenes aureus*.

In spite of the fact that the presence of the serum lessens the efficiency of the disinfectant, it was found that 0.25% carbolic acid disinfected a liberal seeding (6,000,000 per c.c.) of staphylococcus in the presence of serum (previously heated to 60° C.) in less than two weeks at laboratory temperature (Table X). The disinfection was rendered much more rapid at 37° C., and in this case the staphylococci added were killed in two or three days.

TABLE X.

Showing the germicidal effect of dilute solutions of phenol upon staphylococcus pyogenes aureus in the presence of 90—95% blood serum previously heated to 60—70° C.

Experiments made with Method 1.					
Date of exp.	Temperature, degrees centigrade	Concentration of disinfectant parts per 1000	Concentration of blood serum %	Time taken for disinfection in presence of serum	Time taken for disinfection in distilled water
18. 11. 07	20	5	90	2 days	3 hours
	20	2.5	95	9 days	3 days
27. 11. 07	20	5	90	5.5 days	2 hours
	20	2.5	95	more than 12 days	23 hours
27. 3. 08	20	2.5	95	11 days	8 days
	37	2.5	95	30 hours	greater than 8, less than 24 hours
30. 3. 08	20	2.5	95	10.5 days	8 days
	37	2.5	95	50 hours	30 hours

(3) *Experiments with particulate organic matter of various kinds.*

a. Animal charcoal. As might be expected the influence of animal charcoal varies very considerably both according to the nature of the germicide and its condition, viz. whether in solution, e.g. phenol, or in suspension, as in the case of emulsified disinfectants.

50 c.c. of 1% phenol was shaken up with 1 gram. of charcoal and the filtrate was found to contain 0.74%¹. The same amount of charcoal reduced the concentration of 50 c.c. of 5% phenol to 3.85%, the amount removed by the charcoal being nearly proportional to the concentration of the original solution. This suggests that the process is one of adsorption².

¹ Estimated with bromine.

² The first portion of adsorption curves are not uncommonly nearly linear, so that observations confined to a small range of low concentrations show an approximation to proportionality.

With emulsified disinfectants, the proportion removed is much greater. 0.6—0.8 gm. charcoal completely removed the tar-acids from 50 c.c. of an emulsion of disinfectant "A," containing 5 parts in 1000 (2.5 parts per 1000 of tar-acids) so that the filtrate was perfectly clear. In the case of disinfectant "B" 1 gm. charcoal added to 25 c.c. of 5 in 1000 disinfectant (3 per 1000 of tar-acids) gave a filtrate with only a trace of opalescence. In these observations the germicidal value of the filtrates was also directly determined, and it was found that in the case of "B" loss of germicidal power accompanied loss of opacity. Disinfectant "A" contained kresols in addition to higher tar-acids, and in this instance the clear filtrate still possessed one-tenth of its original potency.

The above facts suggest that the removal of an emulsion of tar-acids by animal charcoal might be a case of adsorption. This action of animal charcoal was therefore made the subject of special study and the quantitative relationships between the amount removed and the original concentration determined. The proportion of animal charcoal used was comparatively small, so that a considerable proportion of emulsified tar-acids were left unadsorbed at the end of the experiment. The residual tar-acids after treatment with animal charcoal were estimated. The following method was tested and found to yield sufficiently accurate results.

0.5 gm. animal charcoal (Kahlbaun's purest) was added to 20 c.c. of a series of concentrations of emulsified tar-acids varying from 0.23 to 1.39%. The mixtures were allowed to remain in contact 3 hours in a shaking apparatus. At the end of this period they were filtered and a small proportion (3 to 10 c.c.) of the filtered solution was taken for analysis in each case. The sample was placed in a small separating funnel, acidified with hydrochloric acid, and shaken with ether, in which tar-acids are readily soluble. The watery layer was drawn off and the ether extract was dried by the addition of calcium chloride and withdrawn into a weighed beaker. The calcium chloride was washed with dry ether and the washings added. The ether was evaporated, first on a water-bath and finally on a warm plate at a temperature of 60°—70° C., and the residue weighed.

The results of these experiments, given in Table XI, show that the amount of emulsified tar-acids removed by animal charcoal is not constant. It is at first nearly proportional to the original concentration of tar-acids, but, as this concentration increases, its influence becomes progressively less and the amount adsorbed rapidly reaches a maximum. The maximum amount that the animal charcoal took up was 20% of its own weight.

TABLE XI.

*Adsorption of emulsified tar-acids of different concentrations
by animal charcoal.*

Date of exp.	Concentration of animal charcoal %	Initial concentra- tion of tar-acids %	Final concentra- tion of tar-acids %	Quantity adsorbed by 1 gm. animal charcoal grms.
8. 10. 08	2.5	1.39	0.88	0.202
„	2.5	1.16	0.75	0.200
„	2.5	0.70	0.23	0.185
7. 10. 08	2.5	0.64	0.21	0.169
8. 10. 08	2.5	0.46	0.11	0.144
„	2.5	0.35	0.043	0.122
„	2.5	0.23	0.031	0.080

The amounts adsorbed by 1 gram of charcoal plotted against either the initial or final concentration of the emulsion give points which lie upon a regular curve of the same essential form as has been obtained in the case of other adsorption phenomena¹ (cf. Schmidt (1894); Bayliss (1906)).

b. Dust. The dust was obtained from the tops of cupboards and contained 39 % organic matter and 61 % inorganic residue. The effect of dust in reducing efficiency was tested upon an emulsified disinfectant only.

The results of experiments using method 2 (p. 679), are given in Table XII and it will be seen that with 3 % dust and concentrations of emulsified disinfectant "B" ranging from 1.5 to 4 parts per 1000, there is a reduction of efficiency to about one half, independent of the original concentration of the disinfectant.

With a bacteriological method the observations were necessarily limited to a small range of low concentrations, when, as shown by observations with animal charcoal, the amount of adsorption is approximately proportional to the concentration. This indicates that the action is of a physical and not of a chemical nature.

In an experiment made with *B. typhosus* and the disinfectant "B" at 20° C., using method 1, it was found that when 3 % dust was present a concentration of 1.4 per 1000 was required to disinfect in 15 minutes, whereas in distilled water 0.47 per 1000 was adequate, the effective concentration being reduced to 0.34 of the original. At the same time an experiment was made using 3 % dust which had previously been ignited to free it of all organic matter, and in this case 0.45 parts per 1000 of disinfectant "B" were required for 15 minutes' disinfection,

¹ See this *Journal*, p. 702.

the strength of the disinfectant not being impaired by the addition of the ignited dust. This experiment shows that it is the organic part of dust which alone is responsible for lessening the efficiency of emulsified disinfectants.

TABLE XII.

Effect of presence of dust upon emulsified Disinfectant "B."

B. paratyphosus 20° C. Disinfectant "B."

	Concentration of dust %	Concentration of disinfectant "B," parts per 1000	Time taken for disinfection	Calculated * effective concentration of disinfectant "B," parts per 1000	Relative efficiency (original concentration = 1)
Exp. 31. 10. 07	3	4	0·75 min.	2	0·50
	„	3	2·75 mins.	1·13	0·38
	„	2	6 „	0·92	0·46
	„	1·5	27 „	0·75	0·50
	„	1·0	76·5 „	—	—

* Experiments were made determining time of disinfection for a series of concentrations in distilled water; from the results of these experiments curves were drawn, and the concentrations given in column 4, corresponding to the times in column 3, were determined from the curves.

c. Coagulated horse serum. Coagulated horse serum was used in the form of a very fine precipitate. The serum was coagulated by heat, after dilution with distilled water in such a proportion that the concentration of the suspension was 0·66 %. 15 c.c. of this suspension was mixed with 5 c.c. of disinfectant "B" (2 per 1000). The resulting fluid yielded therefore a suspension of 0·1 gm. proteid in 20 c.c. of an emulsion of disinfectant "B" in the strength of 0·5 in 1000. After centrifuging, the mixture was found to be completely freed from all opacity by the addition of this small quantity of coagulated proteid.

In another experiment 20 c.c. of 0·5 in 1000 disinfectant "B" was completely cleared by the presence of ·07 gm. of the same coagulated serum.

The disinfectant "B" contained 39·2 % of water so that in the above cases the finely divided coagulated albumen had appropriated at least 8 % of its own weight of tar-acids, and this under circumstances which from analogy with the results with animal charcoal (Table XI) must be regarded as the least favourable.

d. Bacteria, dead and alive. A similar phenomenon was observed when suspensions of bacteria, dead or alive, were mixed with solutions

of emulsified disinfectants. While a control tube containing the emulsified disinfectant showed little or no settlement on centrifuging, the tube containing bacteria was cleared of all opacity and disinfectant properties if the relative quantities of disinfectant and bacteria were suitably arranged. For example, when 1 c.c. of an emulsion of *B. paratyphosus* was mixed with 1 c.c. of a solution of disinfectant "B," containing 0.5 part per 1000, the resulting fluid after centrifuging was clear.

In experiments, in which the quantitative relations were studied, the same general results were obtained as with animal charcoal, viz. the amount of tar-acids removed by the bacteria was at first proportional to the concentration and then rapidly reached a maximum. It was found that bacteria could under favourable circumstances remove 39% of their own dry weight of tar-acids from an emulsion.

These facts are capable of explaining the high efficiency of this class of disinfectant and are dealt with in detail in a separate communication¹.

(4) *Influence of the presence of a suspension of faeces upon the various types of disinfectants.*

The bulk of the disinfectants manufactured are destined for the disinfection of excreta, drains, etc. where they have to operate in the presence of more or less faecal matter. It is therefore of interest to learn to what extent the action of various classes of disinfectants may be affected by the presence of faeces.

The fact that the germicidal value of emulsified disinfectants is seriously reduced in the presence of faeces has been pointed out by Kenwood and Hewlett (*loc. cit.*) and Wynter Blyth (*loc. cit.*). Fowler (1906) also made some experiments with a 5% extract of fresh faeces in equal parts of urine and water. The effect upon phenol and emulsified disinfectants of this very dilute watery extract of faeces was found to be negligible. As however all particulate matter was filtered off through paper, Fowler's results do not really bear upon the matter at present under discussion.

The following table (Table XIII) shows the effect upon the efficiency of different classes of disinfectants of the addition of faeces. The concentration of various disinfectants, which killed 6,000,000 per c.c. *B. typhosus* in 15 minutes at 20° C., was determined (a) in distilled

¹ This *Journal*, p. 698.

TABLE XIII.

Disinfectant		Active principle	Concentration required to disinfect about 6,000,000 per c.c. <i>B. typhosus</i> in 15 minutes at 20° C.		Relative efficiency in presence of 3% faeces as reckoned by ratio concentration <i>a</i> concentration <i>b</i>	Remarks
			(a) In distilled water, parts per 1000	(b) In presence of 3% dried faeces, parts per 1000		
(1)	HgCl ₂	5	50	0.10	—
(2)	Commercial disinfectant	HgCl ₂ ...	0.13	1.5	0.09	—
(3)	Commercial disinfectant "Cresol" No. 3	Cresols and higher tar-acids	0.55	6.0	0.09	Contains resin soap as emulsifier. Fine emulsion on dilution with water.
(4)	Commercial disinfectant "B"	Higher tar-acids mostly boiling between 210—260° C.	0.75	4.5	0.17	Contains resin and soft soap as emulsifier. Fine emulsion on dilution with water.
(5)	Commercial disinfectant "Cresol" No. 2	Cresols and higher tar-acids	2.5	8.5	0.29	Contains resin soap as emulsifier. Coarse emulsion on dilution with water.
(6)	Commercial disinfectant "Cresol" No. 1	Cresols and higher tar-acids	5.0	15.0	0.33	Contains resin soap as emulsifier. Coarse emulsion on dilution with water.
(7)	Commercial carbolic acid	91 % Cresols ...	2.2	4.2	0.52	1 grm. almost completely soluble in 100 c.c. water.
(8)	Commercial carbolic acid	95 % Cresols ...	3.2	4.8	0.66	1 grm. almost completely soluble in 100 c.c. water.
(9)	Phenol	8.0	9.25	0.86	—

water, (b) in water containing 3 per cent. by weight of a sample of dried sterile faeces.

The faeces were obtained from an individual upon an ordinary mixed diet. They were dried at 105° C., powdered and passed through a fine sieve and the appropriate quantity weighed out and added to the tubes.

The fact that the presence of organic matter with which it can combine should seriously reduce the disinfecting power of mercuric chloride requires no comment. Phenol, a completely soluble disinfectant, is reduced in value about 10 % by the presence of 3 % faeces. The large effect upon disinfectants, in which the active principles (cresols and tar-acids) are in the form of an emulsion, demands some explanation. The germicidal value of tar-acids is, other things being equal, dependent in some way upon the excellence of the emulsion, and, from the results in Table XIII above, it appears that the amount of interference by faeces

is also related to the fineness of the emulsion; in other words, *the higher the quality of the disinfectant in distilled water due to fineness of emulsion, the more it is interfered with in the presence of particulate organic matter.*

The clue to an interpretation of the deterioration of these emulsified disinfectants in the presence of faeces is afforded by the similar effect of charcoal, dust, coagulated albumen and bacterial suspensions upon this class of disinfectant.

From these observations it appears to be the particulate nature of the organic matter which is responsible for this deleterious influence upon emulsified disinfectants, and the quantitative experiments indicate that the process which takes place is one of adsorption of the fine particles of the emulsion.

Experiments were made to ascertain what quantitative relation existed between the reduction in germicidal value and the original concentration of the emulsion, when the amount of faeces was kept constant. The results of these experiments are set forth in Table XIV and show that the same percentage reduction is produced with concentrations of disinfectant varying between 3 and 7 per 1000.

The results with faeces thus fall into line with those obtained with charcoal, dust and bacteria for similar strengths of emulsion and can also be explained as due to adsorption. Since, however, the emulsified tar-acids in disinfectants "A" and "B" are soluble in olive oil and the sample of faeces used contained 24.3% ether extract, it was conceivable that some portion of the action of faeces might be due to removal of the emulsion by its solution in the fat of the faeces. If this occurred, proportionality between amount removed and the concentration of disinfectant would also be maintained. Accordingly, experiments were made with faeces from which the fat had been extracted and it was found that the action of the extracted faeces was, weight for weight, only slightly less marked than that of the normal faeces (Table XIV). This action of faeces is therefore only due in small part to the solvent action of the contained fat.

It was possible that the loss might be partly or wholly due to a de-emulsifying action of the soluble organic matter present in faeces. Accordingly the dark coloured fluid obtained from a 3 or 6% suspension of faeces in distilled water was tested for any de-emulsifying action upon the disinfectant "B." This fluid was mixed with a dilution of "B," but no settlement was detected either upon standing or after centrifuging.

TABLE XIV.

Quantitative relationship between original concentration of emulsion and depreciation in germicidal value due to presence of faeces.

Date of exp.	Concentration and nature of faeces	Initial concentration of emulsion, parts per 1000	Time taken for disinfection, 5 drops of 24 hrs. culture of <i>B. paratyphoides</i> in 5 c.c., minutes	Calculated effective concentration, parts per 1000	Relative efficiency, initial concentration = 1
(1) 26. 10. 07	3 % dried faeces	6	2.5	1.2	0.20
		5	3	1.15	0.23
		4	13	0.87	0.22
(2) 26. 10. 07	3 % dried fat extracted faeces	5	1.5	1.4	0.28
		4	2.5	1.2	0.30
		3	13	0.87	0.29
(3) 26. 10. 07	2 % dried faeces	7	—	1.00	0.14
		6	—	0.9	0.15
		5	—	0.74	0.15
		4.1	—	about 0.55	0.13

Experiments 1 and 2 were made with Disinfectant "B," Experiment 3 with Disinfectant "A."

The action of faeces in reducing the strength of emulsified disinfectants must therefore be attributed principally to a surface action between the particles of the emulsion on the one hand and those of the faeces on the other, resembling closely the action between the other three types of particulate matter investigated, dust, animal charcoal and coagulated blood serum.

Summary of Chapter VI.

1. The presence of 10 % blood serum reduces the efficiency of 1 % phenol about 12 %. The effect upon emulsified disinfectants is somewhat greater. With mercuric chloride the reduction was much greater, a 0.5 % solution being reduced to from 0.6 to 0.06 of its original value as the concentration of serum was increased from 5 to 30 %.

2. Experiments made with more concentrated serum showed that thirty million staphylococci added to 5 c.c. of the serum containing 0.25 % phenol were killed in less than two weeks at a temperature of 20° C. and in 30 to 50 hours at a temperature of 37° C.

3. The presence of particulate organic matter—animal charcoal, dust, finely precipitated coagulated albumen, bacteria, and faeces—affect

the germicidal value of emulsified disinfectants containing tar-acids to a much greater extent than that of solutions of phenol.

4. The whole of the emulsified tar-acids can be removed by a suitable addition of any of the above forms of particulate organic matter.

5. The amount of tar-acids removed by animal charcoal from an emulsified disinfectant is at first proportional to the original concentration of tar-acids, but, as this concentration increases, the proportion removed rapidly diminishes. The amounts removed by a given quantity of charcoal from different strengths of emulsion increase at first rapidly and then more and more gradually until, with an emulsion of 1.4% tar-acids, the maximum is nearly reached. The curve drawn from the observations, tabulated in Table XI, presents the usual form of an adsorption curve (see this *Journal*, p. 702, Fig. 1).

6. The removal of an emulsion of tar-acids by bacteria obeys the same quantitative laws as obtain in the case of animal charcoal.

7. The removal of tar-acids by dust was only investigated over a limited range of initial concentration and was found to take place in accordance with the same general law.

8. The effect of a 3% suspension of finely powdered dried faeces upon the efficiency of phenol was to reduce it about 10%; upon commercial cresols a reduction of 30 to 50% was produced, depending upon the completeness with which the preparation dissolved. The freer the sample was from higher and insoluble homologues, the less depreciation in the original value occurred.

9. The effect upon various emulsified disinfectants containing higher tar-acids was to reduce their efficiency to a value varying from $\frac{1}{3}$ rd to $\frac{1}{11}$ th of the original. The finer emulsions were more seriously reduced in value than the coarser ones.

10. Within the limits of concentration of disinfectant employed, the amount of emulsion removed from the liquid by powdered faeces was nearly proportional to the concentration of disinfectant. In this case, as with the other forms of particulate organic matter, the removal was shown to be principally due to adsorption of the emulsion upon the surfaces of the fine particles.

CHAPTER VII. A METHOD OF STANDARDISING DISINFECTANTS IN THE PRESENCE OF FAECES.

It might be imagined that faeces would prove an impossible material to be included in a standard test on account of inconstancy in

composition. This has been contended by Wynter Blyth (*loc. cit.*) and Somerville and Walker (*loc. cit.*), and is certainly true if the faeces are employed in their natural condition, but has not been found by us to be the case if they are dried and powdered. Our experience on the contrary has shown that, when dried and ground to a fine powder, faeces from different individuals upon an ordinary mixed diet display a surprising uniformity with regard to the extent to which they influence the germicidal power of a disinfectant.

The particulate matter in human faeces consists largely of bacteria and portions of undigested food, together with varying quantities of fat and other bodies soluble in ether. In addition, a considerable amount of the pigment hydrobilirubin is present in an insoluble form, combined with calcium. These together account for about 70 % of the total dry weight. In the preceding chapter we have shown that the reduction of the efficiency of emulsified disinfectants by faeces is caused by the solid particles contained therein and is principally due to adsorption, hence depending as much upon the physical condition of the faeces as upon their chemical composition.

The following experiments (see Tables XV and XVI) illustrate the effect of 15 different samples of faeces, dried at 105° C. and ground to a fine powder, upon the germicidal value of emulsified disinfectants. In the experiments with *B. typhosus* (Table XVI) the variation caused by the different samples of faeces is seen to be within the error of the method, for the divergence between the results obtained upon the same day with faeces from different individuals was not greater than those obtained with the same sample of faeces upon different days.

TABLE XV.

Emulsified disinfectant "B" with various samples of faeces.

Disinfection of about 6,000,000 *B. paratyphosus* per c.c. (5 drops from a 24 hours' culture at 37° C.).

Sample of faeces	Concentration of "B" required for complete disinfection in 15 mins. at 20° C. in presence of 3% faeces, parts per 1000
I	5.5
II	5.5
III	5.0
IV	4.5
V	5.0

TABLE XVI.

*Emulsified disinfectant "B" with various samples of faeces.*Disinfection of about 6,000,000 *B. typhosus* per c.c. (5 drops from a 24 hours' culture at 37° C. in 5 c.c.).

Concentration of "B" required for complete disinfection in 15 minutes at 20° C., in presence of 3 0/100 faeces, parts per 1000.

Sample of faeces	Date of exp.:						
	21. 3. 08	28. 3. 08	29. 4. 08	5. 5. 08	7. 5. 08	8. 5. 08	9. 5. 08
II	—	—	—	—	—	4.0	—
V	—	—	—	—	—	—	4.5
VI	—	—	—	4.8	—	—	—
VII	—	—	4.4	—	—	4.0	—
VIII	—	—	—	—	—	4.6	—
						more than	
C	5.2	4.8	—	4.3	4.0	6	—
D	—	—	—	—	—	4.0	—
E	—	—	—	—	4.6	—	—
F	—	—	—	—	—	—	4.3
G	4.4	4.1	—	—	—	—	—
H	4.1	—	—	—	—	—	4.9
I	—	—	—	4.5	4.3	—	4.3

TABLE XVII.

Mercuric chloride with various samples of faeces.

Exp. 23. 7. 08	Samples of faeces	Concentration of mercuric chloride required for disinfection of about 6,000,000 per c.c. <i>B. typhosus</i> in presence of 3 0/100 faeces at 20° C. in 15 minutes, parts per 1000	Nos. expressing concentration of mercuric ions
	VII	1.0	63
	VIII	1.0	63
	IX	1.5	68
	II	1.5	68
	I	2.0	71
	I	2.0	71

The same uniformity of effect was apparently not maintained when the experiments were made with mercuric chloride, but here again the discrepancy is really within the experimental error. As shown in Table XVII the concentration necessary to disinfect 6,000,000 per c.c. *B. typhosus* in 15 minutes varied between 1 and 2 parts per 1000. This is not however of material significance, because the error of determining the germicidal value of mercury in this concentration is about 100%, owing to the fact that, although the concentration of mercuric

chloride changes from 1 to 2, the concentration of mercuric ions only varies as 63 to 71.

Description of the method employed.

The essential features of the method which we have been in the habit of using are those of the Rideal-Walker process, but a constant time, 30 minutes, is allowed for the disinfectant to act. The introduction of the faeces somewhat increases the experimental error which if we exclude one aberrant result in Table XVI is now less than 20%, whereas in distilled water (see p. 665) we could rely upon an error not exceeding 10%.

The faeces used were dried first in a water bath and subsequently at 105° C., ground to a fine powder, and passed through a fine sieve with a mesh of 130 to the inch. Quantities of 0.15 grm. were weighed out and placed in test tubes. To each test tube 2.5 c.c. distilled water was added and the tube sterilised in the autoclave (10 minutes at 120° C.). The tubes were covered with indiarubber caps and kept in jars with greased lids to prevent evaporation.

At the time of the experiment different amounts of a suitable dilution of the disinfectant were added to each tube together with enough distilled water to make the total volume up to 5 c.c. The tubes then contained different concentrations of the disinfectant in question in the presence of 3% faeces¹. The tubes were inoculated and sampled in exactly the same way as when the test was made in distilled water (see p. 665).

In the case of an unknown disinfectant one or two preliminary trials are necessary to ascertain a suitable range of concentrations to give a useful result. The exact procedure may be made clearer by an actual example.

Six tubes each containing 0.15 gr. faeces in 2.5 c.c. of distilled water having been prepared, to each is added varying quantities of 2% disinfectant and water as in Table XVIII below, so arranged that the tubes contain 0.5 c.c. of a series of concentrations of disinfectant in the presence of 3% faeces. In this experiment the approximate concentration necessary to disinfect in 15 minutes has been determined previously, and the actual concentration required to disinfect in the time is found to be between 4 and 4.6 parts per 1000.

¹ Three per cent. faeces was chosen as representing the amount of solid matter present if a liquid stool (containing 10% solids) were mixed with twice its volume of the disinfectant.

An exactly similar experiment with pure phenol is simultaneously made, so that the carbolic acid coefficient of the disinfectant can be directly determined.

TABLE XVIII.

Faeces I. 3 $\frac{0}{10}$, and a constant number of *B. typhosus* per c.c.

	No. of c.c. of 2 $\frac{0}{10}$ disinfectant	No. of c.c. distilled water added	Total volume added, c.c.	Concentration of disinfectant in the tube, parts per 1000	Result of experiment + = acid formation in test cultures* in glucose broth	
Exp. 9. 5. 08	0.9	1.6	2.5	3.6	+	+
	1.0	1.5	2.5	4.0	+	+
	1.15	1.35	2.5	4.6	-	-
	1.3	1.2	2.5	5.2	-	-
	1.5	1.0	2.5	6.0	-	-
	1.7	0.8	2.5	6.8	-	-

* Test cultures were always made in duplicate.

This method in one way departs from the methods of practical disinfection. A disinfectant is in practice added to a mixture of organic matter and bacteria; in the method above the disinfectant is previously mixed with the organic matter and the bacteria are added subsequently. It was conceivable that the latter order of mixing might impose a harder test upon the disinfectant than was necessary. Comparative experiments were therefore made using either method, in the one case the test was carried out as already described, in the other case the bacteria were introduced into the solution of faeces and the disinfectant added to this mixture. No significant difference was found between experiments using the two methods.

	Disinfectant added before culture. Concentration necessary for 15 minutes disinfection, parts per 1000	Disinfectant added after culture. Concentration necessary for 15 minutes disinfection, parts per 1000
Exp. 16. 7. 07	5.0	4.4
Exp. 19. 7. 07	4.8	4.8

We are not particularly enamoured with the method of testing a disinfectant in the presence of three per cent. dried faeces. In many respects it departs from the ideal, but by its means the germicidal value of a disinfectant can be approximately measured under conditions such as it may not unlikely encounter in practice. We realise that disinfectants are usually employed in a very casual way and quite as commonly as deodorizers as with a view to the sterilisation of the germs of disease. But there are times when the medical man or sanitarian

wants to know what reliance he can place upon this or that disinfectant for a particular purpose, and we think that a determination of the value of a disinfectant made in the presence of three per cent. faeces will, for the majority of purposes, be more valuable than when made in distilled water. Everyone, we presume, would agree as to the absurdity of forming an estimate of the utility of an oxidising disinfectant, such as permanganate of potash, by determining its germicidal value upon a few bacteria in distilled water, or to accept the statement that 1 in 10,000 parts of mercuric chloride would be a reliable disinfectant for pus, because this concentration will suffice in distilled water to destroy 6,000,000 *B. typhosus* per c.c. in 15 minutes at 20° C. Except on a few occasions, for surgical purposes, a disinfectant will have to act in the presence of more or less particulate organic matter, and we have chosen that form which will be present in one of the common cases for which disinfectants are employed.

Our observations have clearly shown that just as the value of oxidisers will, from their nature, be depreciated in the presence of oxidisable matter other than the bacteria, and mercurial preparations by the presence of proteid or other material with which mercury combines, so emulsified disinfectants from their very nature will be diminished in value by the adsorption of the emulsion of tar-acids upon a great variety of particles of organic matter, if such be present.

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A COMPARISON OF THE POWER OF A GERMICIDE
EMULSIFIED OR DISSOLVED, WITH AN INTERPRE-
TATION OF THE SUPERIORITY OF THE EMULSIFIED
FORM.

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HENLE (1889) found that "Creolin," an emulsion of tar acids, was a more efficient disinfectant than could be explained from observations upon each of its constituents separately, and concluded that the extra efficiency was connected with the emulsified form. Henle's conclusions do not appear to us to be necessarily justified by his observations.

Rideal and Walker (1903, p. 425) state that an emulsion of Trikresol is equal in germicidal power to a solution three times as concentrated, but give no details.

We have compared the germicidal value of higher tar acids when emulsified in water and when dissolved in alcohol. The spores of *B. subtilis* were chosen, because alcohol alone was found to have no action upon them within the time limits employed.

A suspension of an old sporing culture of *B. subtilis* was made in water and heated for some minutes to 80° C. By a preliminary plating experiment an amount of spores suitable for enumeration experiments was determined, and this amount was added to two tubes containing 2.5% tar acids in solution in alcohol and emulsified in water respectively. The tubes were placed in a water bath at 20° C. and a definite quantity removed from each at known intervals of time, and plated. After 24 hours' incubation the numbers of colonies on the plates were counted.

The numbers of colonies formed a regularly decreasing series in both cases, but diminished more rapidly in the case of disinfection by tar acids in the form of an emulsion (see Table I).

Disinfection has been shown by Madsen and Nyman (1907) and by one of us (H. C. 1908) to proceed according to the equation

$$\frac{1}{t_2 - t_1} \log \frac{n_1}{n_2} = K,$$

where t_1 and t_2 are any two times, and n_1 and n_2 the numbers of surviving organisms in unit volume of the liquid at times t_1 and t_2 respectively, and K a constant depending on the particular organism and disinfectant, the concentration of the latter, and the temperature.

This constant is therefore an expression for the velocity of the reaction.

From the number of bacteria surviving after various intervals of time (see Table I, col. 4) two values of K were calculated (see col. 5), the ratio of which expresses the relative velocity of the disinfection by the same concentration of tar acids in the two conditions.

The mean value for K was 0.049 for the emulsion and 0.0066 for the solution, so that coal tar acids in the above concentration disinfected

TABLE I.

Spores of B. subtilis. 20° C.

Disinfectant	Time elapsing, minutes	Amount of sample	Mean no. of bacteria present in 1 drop disinfecting mixture	K , assuming reaction to be in accordance with the equation $-\frac{dn}{dt} = Kn$ ($K = \frac{1}{t_2 - t_1} \log \frac{n_1}{n_2}$)
Exp. 29. 2. 08				
2½ % tar acids emulsified in water	1	1 drop	2750	taken as an initial value of $n (=n_1)$ in calcu- lating values of K .
	4.5	1 „	1804	0.052
	20	1 „	373	0.046
			Mean value of K 0.049	
2½ % tar acids dissolved in alcohol	1	1 „	1750	taken as an initial value of $n (=n_1)$ in calcu- lating values of K .
	70	1 „	607	0.0067
	121	1 „	393	0.0054
	230	1 „	32	0.0077
			Mean value of K 0.0066	

7.5 times¹ as quickly in the form of an emulsion as when dissolved in alcohol.

The explanation of this increased efficiency of a germicide in the emulsified form demands some consideration. Fowler (1907) suggested that there is a mutual attraction between the particles of an emulsion and the bodies of bacteria. He was led to this conclusion from the examination of stained microscopical preparations containing mixtures of bacteria and emulsions; he then observed bacteria with particles of disinfectant adhering to them. On repeating Fowler's observations it was found that methylene blue, in addition to staining the particles, de-emulsified the disinfectant, so that conclusions drawn from appearances seen after its addition are not free from fallacy.

In order to arrive at an understanding of what takes place, we observed mixtures of living bacteria (*B. paratyphosus*) and an emulsified disinfectant, under a magnification of 1100 diameters, using a dark background and side illumination. Under these circumstances the bacteria and the particles of the disinfectant appear as shining bodies and their relation to one another can be easily observed.

The particles of the emulsion, and to a lesser degree the bacteria, exhibited active Brownian movement. The bacteria, which were considerably larger than the mean diameter of the emulsified particles, were seen to be continually bombarded by the latter. The bacteria were thus frequently brought into intimate contact with particles of tar acid somewhat smaller than themselves, but whether any portion was left remaining upon them could not be directly observed. Nothing of the nature of an agglutination of the particles upon the bacteria was seen, although an occasional temporary attachment was noticed.

Nevertheless, whereas the emulsified disinfectant alone showed a multitude of small shining bodies, an emulsion of equal concentration to which bacteria had been added showed a great reduction in the number of these bodies, so that many of the emulsified particles of the disinfectant had been appropriated by the bacteria.

This appropriation by bacteria of particles of tar acids from an emulsion was readily demonstrated by mixing together a suspension of bacteria and an emulsion of tar acids, allowing them to remain in contact a short time, and subsequently centrifuging. Under these circumstances the mixture was completely cleared of all opacity when

¹ This does not mean that the disinfectant in the emulsified form was, weight for weight, 7.5 times as efficient, see "An Investigation of the Laws of Disinfection," by H. Chick, this *Journal*, pp. 117—132.

the relative quantities of tar acids and bacteria were suitably arranged. A control tube containing the emulsion showed very little settlement on centrifuging.

The suspension of bacteria (*B. paratyphosus*) employed contained 0·025 gram. dry weight of bacteria per c.c. When 1 c.c. of this bacterial suspension was mixed with 1 c.c. of an emulsion containing 0·6 in 1000 of tar acids, so that the 2 c.c. of mixture possessed a concentration of 0·3 per 1000, a perfectly clear solution was obtained after centrifuging, i.e. 0·025 gram. bacteria united with 0·0006 gram. of tar acids, which is more than 2% of its own weight.

In order to gain some further insight into the nature of the process a series of quantitative experiments were undertaken using a constant weight of bacteria and emulsions of varying concentration. *Staphylococcus pyogenes aureus* was employed. The cocci were three times well washed with water and separated by means of a centrifuge. The final bacterial suspension contained 0·0785 gram. dry weight of cocci in 1 c.c.; 2 c.c. of this suspension were added to a series of tubes each containing 6 c.c. of an emulsion of tar acids, ranging in strength from 0·15 to 0·89%. The contents were well mixed and, after two hours, centrifuged. A measured quantity of the supernatant liquid was removed and acidified, the tar acids extracted with ether, and determined (see this *Journ.* p. 683).

The results are set out in Table II, and show that the emulsified tar acids removed by one gramme of staphylococci is at first roughly proportional to the original concentration of tar acids, but, as this concentration increases, its influence becomes progressively less and the amount removed rapidly tends to a maximum.

TABLE II.

Adsorption of emulsified tar acids by bacteria.

	Organism employed	Concentration of bacteria dry weight %	Initial concentration of tar-acids %	Final concentration of tar-acids %	Amount adsorbed by 1 gram. dry weight bacteria, grms.
Exp. 19. 9. '08	Morgan's	1	0·714	0·414	0·300
	Bacillus	1	0·178	0·094	0·084
Exp. 28. 9. '08	<i>Staphylococcus pyogenes aureus</i>	1	0·892	0·496	0·396
		1	0·594	0·228	0·366
		1	0·446	0·164	0·282
		1	0·298	0·086	0·212
		1	0·148	0·072	0·076

The results with bacteria are indeed similar to those obtained by us with animal charcoal (Table XI, this *Journ.* p. 684), but one gramme dry weight of bacteria removes, under similar conditions, about twice as much of the emulsion as was removed by the preparation of animal charcoal employed, viz. 39·6% of its own weight.

We found that the cocci lost about 50% of water on drying at 110° C. so that weight for weight bacteria and animal charcoal happen to be about equally effective. This similarity between the two processes is strikingly demonstrated if the figures for the amounts removed by one gramme of bacteria and charcoal respectively are both plotted against the original concentration of the emulsion. This has been done in Fig. 1, where the circles represent the amounts of tar acids adsorbed by one gramme charcoal and the crosses that removed by one gramme of bacteria in their natural condition. It will be seen that the curve drawn for the adsorption by charcoal would do almost equally well for that by the cocci.

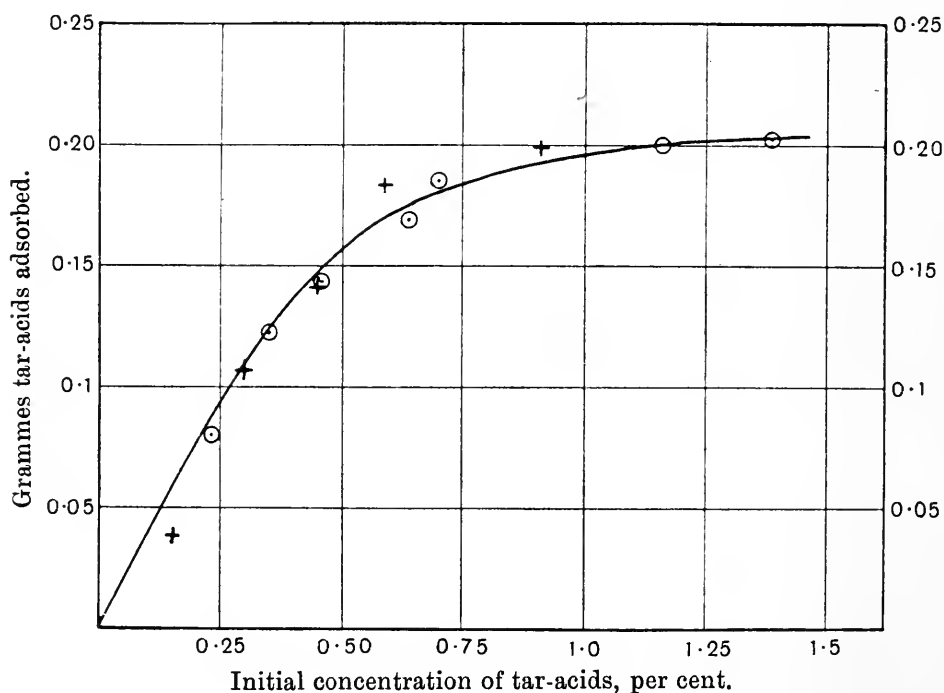


Fig. 1.

From these results there is little doubt that the removal of an emulsion of tar acids by bacteria is, in the first instance, a process of adsorption and not a chemical combination, and that disinfectants of this class possess superior efficiency, because owing to this adsorption

the bacteria rapidly become surrounded by the disinfectant in much greater concentration than exists throughout the liquid. As has been pointed out elsewhere, however (this *Journ.* p. 689), the same property of adsorbing the particles of the emulsion is possessed by most organic particles, in consequence of which the germicidal value of this class of disinfectant is greatly deteriorated by the presence of particulate organic matter other than the bacteria which it is desired to destroy.

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